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(54) Title: TISSUE SPECIFIC HYPOXIA REGULATED THERAPEUTIC CONSTRUCTS		
(57) Abstract <p>Methods and compositions relating to chimeric genes containing (i) a tissue-specific promoter and (ii) a hypoxia response enhancer element, both of which are operably linked to a selected gene, such as a reporter gene, therapeutic gene (e.g., bcl-2, NOS, catalase and SOD), or deleterious gene are disclosed. Expression of the selected gene is enhanced in the target tissue under hypoxic conditions, such as conditions encountered during episodes of ischemia and reperfusion. The methods and compositions may be used as therapeutics and/or diagnostics.</p>		

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TISSUE SPECIFIC HYPOXIA REGULATED
THERAPEUTIC CONSTRUCTS

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FIELD OF THE INVENTION

This invention relates to chimeric genes (e.g., carried on expression vectors) containing therapeutic genes whose expression is under the control of tissue specific and hypoxia response enhancer elements.

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REFERENCES

- Ascadi, G., *et al.*, *Nature* 352:815 (1991b).
- Ascadi, G., *et al.*, *New Biology* 3:71 (1991a).
- 15 Atkins, C.E., *et al.*, *J. Am. Vet. Med. Assoc.* 201:613-618 (1992).
- Ausubel, F.M., *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley and Sons, Inc., Media PA.
- Berkner, K.L., *BioTechniques* 6:616 (1988).
- Bisphopric, *et al.*, *J. Clin. Invest.* 80:1194 (1987).
- 20 Breakefield, X.O., and DeLuca, N.A., *New Biol.* 3:230 (1992).
- Bredt, D.S., *et al.*, *Nature* 351:714-718 (1991).
- Buttrick, P.M., *et al.*, *Circ. Res.* 70:193-198 (1992).
- Buttrick, P.M., *et al.*, *Circ. Res.* 72:1211-1217 (1993).
- Chatterjee, J., *et al.*, *Science* 258:1485 (1992).
- 25 Chomczynski, P., and Sacchi, N., *Anal. Biochem.* 162:156-159 (1987).
- Christiano, R.J., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 90:212 (1993).
- Clair, D.K.S., *et al.*, *Cancer Res.*, 51:939 (1991).
- Cleveland, J.L., and Ihle, J.N. *Cell* 81:479-482 (1995).
- Dabareiner, R.M., *et al.*, *Am. J. Vet. Res.* 54:1683-1692 (1993).
- 30 Dayhoff, M.O., ATLAS OF PROTEIN SEQUENCE AND STRUCTURE, suppl. 3, National Biomedical Research Foundation, Washington, D.C. (1978).
- Doolittle, R.F., OF URFs AND ORFs, University Science Books (1986).
- Flugelman, *et al.*, *Circulation* 82:2217 (1990).
- Fox, P.R., *et al.*, *Am. J. Vet. Res.* 54:563-569 (1993).
- 35 Franz, W-M, *et al.*, *Circ. Res.* 73:629 (1993).
- Freese, A., *et al.*, *Biochem. Pharm.* 40:2189 (1990).

- Frei, B., *Am. J. Med.* 97 suppl 3A:5s-13s (1993).
- Friedman, J.M., *et al.*, *Mol. Cell Biol.* 6:3791-3797 (1986).
- Fujisawa, H., *et al.*, *J. Neurochem.* 63:140 (1994).
- Fukamizu, A., *et al.*, *Biochem. Biophys. Res. Commun.* 199:183 (1994).
- 5 Giallongo, A., *et al.*, *Eur. J. Biochem.* 214:367 (1993).
- Gorechi, *et al.*, *Free Radic. Res. Commun.* 12-13:401 (1991).
- Gottlieb, R.A., *et al.*, *J. Clin. Invest.*, 94:1612-1628 (1994).
- Graham, F.L., and Prevea, L., in METHODS IN MOLECULAR BIOLOGY, Vol. 7 (Murray, E.J., Ed.) (Humana, Clifton, NJ) pp. 109-127 (1991).
- 10 Grunhaus, A. and Horowitz, M.S., *Semin. Virol.*, 3:237-252 (1992).
- Gulick, J., *et al.*, *J. Biol. Chem.* 266:9180-85 (1991).
- Gustafson, T.A., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 84:3122-3126 (1987).
- Hansen, P.R. and Stawaski, G., *Cardiovasc. Res.*, 28:565-569 (1994).
- Heacock, C.S. and Sutherland, R.M. *Br. J. Cancer* 62:217-228 (1990).
- 15 Hertz, J., and Gerard, R.D., *Proc. Natl. Acad. Sci. U.S.A.*, 90:2812-2816 (1993).
- Hockenbery, D.M., *et al.*, *Nature* 348:334-336 (1990).
- Hockenbery, D.M., *et al.*, *Cell* 75:241 (1993).
- Hope, T.J., *et al.*, *J. Virol.* 66:1849 (1992).
- Inoue, A., *et al.*, *J. Biol. Chem.* 264:14954-14959 (1989).
- 20 Jaffe, H.A., *et al.*, *Nat. Genet.* 1:374 (1992).
- Jahroni, N., and D.C. Lynch, *Mol. Cell. Biol.* 14:999-1008 (1994).
- Jones, N., and Shenk, T., *Cell* 16:683 (1979).
- Karin, M., and Herrlich, P., in GENES AND SIGNAL TRANSDUCTION IN MULTISTAGE CARCINOGENESIS (Colburn, N.H., Ed.) Marcel Dekker, New York, NY, pp. 415-440 (1989).
- 25 Kasahara, N., *et al.*, *Science* 266:1373 (1994).
- Kass-Eisler, *et al.*, *Proc. Natl. Acad. Sci.* 90:11498-11502 (1993).
- Kennedy, P.G. and Steiner, I. *Q.J. Med.* 86:697-702 (1993).
- Kirshenbaum, L.A., *et al.*, *J. Clin. Invest.* 92:381 (1993).
- Kitsis, R., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88:4138 (1991).
- 30 Kourembanas, S., *et al.*, *J. Clin. Invest.* 92:99 (1993).
- Kwok, T.T., and Sutherland, R.M., *JNCI* 81:1020-1024 (1989).
- Laderoute, K.R., *et al.*, *Int. J. Cancer* 52:428-432 (1992).
- Lantz, G.C., *et al.*, *Am. J. Vet. Res.* 53:1594-1598 (1992).
- Leclere, G., *et al.*, *J. Clin. Invest.* 90:936 (1992).

- Lefer, *et al.*, *Circulation* 88:1779-1787 (1994).
- Lin, H., *et al.*, *Circulation* 82:2217 (1990).
- Lord, E.M., *et al.*, *J. Cancer Res.* 53:5721-5726 (1993).
- Luke, M.C., *et al.*, *J. Androl* 15:41 (1994).
- 5 Madan, A., *et al.*, *Proc. Natl. Acad. Sci.* 90:3928 (1993).
- Mahdavi, V., *et al.*, *Proc. Natl. Acad. Sci.* 81:2626 (1984).
- Malim, M.H., *et al.*, *J. Exp. Med.* 176:1197 (1992).
- Malin, M.H., *et al.*, *Cell* 58:205 (1989).
- 10 Marci, P., *et al.*, *Hum. Gene Ther.* 5:175 (1994).
- Miller, A.D., *Hum. Gene Ther.* 1:5 (1990).
- Miller, *et al.*, *Vet. Clin. North Am. Anim. Pract.* 19:87-102 (1989).
- Minty, A., and Kedes, L., *Mol. Cell Biol.* 6:2125-2136 (1986).
- Molkentin, J.D., *et al.*, *Mol. Cell Biol.* 144:947-4957 (1994).
- 15 Morishita, R., *et al.*, *J. Clin. Invest.* 91:2580 (1993).
- Mullis, K.B., *et al.*, U.S. Patent No. 4,683,195, issued 28 July 1987.
- Mullis, K.B., U.S. Patent No. 4,683,202, issued July 28, 1987.
- Murtha, P., *et al.*, *Biochem.* 32:6459 (1993).
- Muscat, G.E.O, and Kedes, L., *Mol. Cell Biol.* 7:4089-4099 (1987).
- 20 Nabel, E.G., *et al.*, *Science* 249:1285 (1990).
- Nakane, M., *et al.*, *FEBS Lett.* 316:175 (1993).
- Pennica, D., *et al.*, *Nature* 312:724-729 (1984).
- Peshavaria, M., and Day, I.N.M., *Biochem. J.* 275:427-433 (1991).
- Quantin, B., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89:2581 (1992).
- 25 Reimer, D.L., *et al.*, *Genomics*, 21:325 (1994).
- Rosenberg, M.E. and Paller, M.S. *Kidney International*, 39:1156-1161 (1991).
- Rosenfeld, M.A., *et al.*, *Science* 252:431 (1991).
- Rosenfeld, M.A., *et al.*, *Cell*, 68:143-155 (1992).
- Rossi, J.J., and Sarver, N., *Adv. Exp. Med. Biol.* 312:95 (1992).
- 30 Sambrook, J., *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, Second Edition, Cold Spring Harbor Laboratory (Cold Spring Harbor, NY) (1989).
- Sasaoka, T., *et al.*, *Brain Res. Mol. Brain Res.* 16:274 (1992).
- Schulz, G.E., *et al.*, PRINCIPLES OF PROTEIN STRUCTURE, Springer-Verlag New York Inc. (1979).

- Scott-Moncrieff, J.C., *et al.*, *J. Am. Vet. Med. Assoc.* 201:1553-1558 (1992).
- Semenza, G.L., and Wang, G.L., *Mol. Cell Biol.* 12:5447-5454 (1992).
- Seto, M., *et al.*, *EMBO J.* 7:123 (1988).
- Shirai, T., *et al.*, *Nature* 313:803-806 (1985).
- 5 Smith, E.F., *et al.*, *Am. J. Physiol.* 255:H1060-H1068 (1988).
- Stratford-Perricaudet, L.D., *et al.*, *J. Clin. Invest.* 90:626 (1992a).
- Stratford-Perricaudet, L.D., *et al.*, *Bone Marrow Transplant* 9(suppl. 1):151 (1992b).
- Subramaniam, A., *et al.*, *J. Biol. Chem.* 268:4331-4336 (1993).
- Sullenger, B.A., *et al.*, *J. Virol.* 65:6811 (1991).
- 10 Sullivan, K.E., *et al.*, *Vet. Surg.* 22:343-350 (1993).
- Takenaka, M., *et al.*, *J. Biol. Chem.* 264:2363-2367 (1989).
- Takiguchi, M., *et al.*, *J. Biol. Chem.* 266:9186 (1991).
- Thornton, J.D., *et al.*, *J. Mol. Cell Cardiol.* 25:311 (1993).
- Titus, D.E., ed., PROMEGA PROTOCOLS AND APPLICATIONS GUIDE, Second Edition,
- 15 Promega Corporation (Madison, WI) (1991).
- Tsujimoto, Y., *et al.*, *Proc. Natl. Acad. Sci.* 83:5214-18 (1986).
- Vibert, M., *et al.*, *Eur. J. Biochem.* 181:33 (1989).
- Wagner, E., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89:6099 (1992a).
- Wagner, E., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89:7934 (1992b).
- 20 Webster, K.A., and Bishopric, N.H., *J. Mol. Cell Cardiol.* 24:741-751 (1992).
- Webster, K.A. and Kedes, L., *Mol. Cell Biol.* 10:2402-2406 (1990).
- Webster, K.A., *et al.*, *J. Biol. chem.* 268:16852-16858 (1993).
- Williams, G.T. and Smith C.A. *Cell*, 74:777-778 (1993).
- Wilson, D.V., and Stick, J.A., *Am. J. Vet. Res.* 54:442-448 (1993).
- 25 Wolf, A., *et al.*, *Science* 247:1465 (1990).
- Wu, G.Y., *J. Biol. Chem.* 266:14338 (1991).
- Youker, *et al.*, *J. Clin. Invest.* 89:602-609 (1992).
- Yung, W.K., *Curr. Opin. Oncol.* 6:235-239 (1994).
- Zhang, L.X., *et al.*, *Neuroreport*, 3:700 (1992).
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BACKGROUND OF THE INVENTION

Each year, over a half-million Americans die from heart attacks. Even more -- close to 700,000 -- have non-fatal heart attacks. For these surviving victims, a portion of the heart is usually damaged irreparably. Such cell death of cardiac tissue, called myocardial infarction,

is due in large part to tissue damage caused by ischemia and/or ischemia followed by reperfusion.

Similar ischemic damage may occur in many other tissues when the blood supply to the tissue is reduced or cut off. Stroke, deep vein thrombosis, pulmonary embolus, and renal failure are examples.

Surviving victims of ischemic episodes, such as heart attacks, are at substantially greater risk for subsequent episodes of ischemia, which in many cases prove debilitating or fatal. Thus, it would be desirable to have therapeutic methods and compositions by which survivors of heart attacks and other types of ischemic insults could lower the risk of tissue damage due to recurrent ischemic/reperfusion episodes.

SUMMARY OF THE INVENTION

In one aspect, the invention includes a method for reducing ischemic injury to a cell exposed to hypoxic conditions. The method includes introducing into the cell a chimeric gene containing a hypoxia response element, a therapeutic gene, and a tissue-specific promoter operably linked to the therapeutic gene to control transcription of the therapeutic gene in the cell, where the element is effective to modulate expression of the therapeutic gene. Exposing the cell to hypoxic conditions enhances expression of the gene and expression of the gene is effective in reducing ischemic injury to the cell. The method may be applied to, for example, cardiac cells using a cardiac-specific promoter, kidney cells using a kidney-specific promoter, brain cells using a brain-specific promoter, and vascular endothelium cells using a vascular endothelium-specific promoter. The hypoxia response element may be selected from, for example, the erythropoietin HRE element (HREE1), muscle pyruvate kinase (PKM) HRE element, β -enolase (enolase 3; ENO3) HRE element, endothelin-1 (ET-1) HRE element and metallothionein II (MTII) HRE element. The therapeutic gene may be selected from, for example, nitric oxide synthase (NOS), B-cell leukemia/lymphoma 2 (bcl-2), superoxide dismutase (SOD) and catalase. In a preferred embodiment, the promoter is heterologous to said element.

In another aspect, the invention includes a chimeric gene, containing a hypoxia response element, a tissue-specific promoter heterologous to the element, and a therapeutic gene. The promoter is operably linked to the therapeutic gene and the element is effective to modulate expression of the therapeutic gene. The method may be used with a variety of cell types and corresponding promoters, for example, as identified above. Suitable cardiac-specific promoters include the α -MHC_{3.5} promoter, α -MHC₆ promoter, and human cardiac actin

promoter. Suitable kidney-specific promoters include the renin promoter. Suitable brain-specific promoters include the aldolase C promoter and the tyrosine hydroxylase promoter. Suitable vascular endothelium-specific promoters include the Et-1 promoter and vonWillebrand factor promoter. Hypoxia response enhancer element useful with the method include HREE1,
5 PKM HRE element, ENO3 HRE element and ET-1 HRE element. Exemplary therapeutic genes useful with the method include NOS, Bcl-2, SOD and catalase.

Another aspect of the present invention includes the above-described chimeric gene carried in an expression vector. The expression vector may be a plasmid, adenovirus vector, retrovirus vector, or the like.

10 In still another aspect, the invention includes a chimeric gene which contains a hypoxia response element, a tissue-specific promoter heterologous to the element, and a deleterious gene. The promoter is operably linked to the deleterious gene, and the element is effective to modulate expression of the deleterious gene. Suitable promoters include tumor-specific promoters, such as alpha fetoprotein (AFP) promoter. Suitable hypoxia response elements are
15 as articulated above. Deleterious genes useful in this aspect include a viral thymidine kinase gene (tk), such as the herpes simplex virus (HSV) tk, and tumor necrosis factor (TNF).

In a related aspect, the invention includes a method of causing injury to a cell exposed to hypoxic conditions. The method includes introducing into the cell a vector containing a hypoxia response element, a deleterious gene, and a tissue-specific promoter operably linked
20 to the gene and capable of controlling transcription of the gene in the cell. Exposing the cell to hypoxic conditions enhances expression of the gene, and expression of the gene is effective to cause injury to the cell. Promoters useful with this method include tumor-specific promoters such as the AFP promoter. Specific hypoxia response elements and deleterious genes useful with the method are also as identified above.

25 The invention also includes a chimeric gene which contains a hypoxia response element isolated from the metallothionein II promoter (e.g., an HRE contained in a fragment having the sequence represented as SEQ ID NO:35), a promoter and a heterologous gene. In one general embodiment, the heterologous gene is a therapeutic gene, as described above. In another general embodiment, the heterologous gene is a deleterious gene as described above (e.g., a
30 DNA sequence encoding tumor necrosis factor).

The invention further includes a method of causing injury to a cell exposed to hypoxic conditions. The method includes introducing into the cell a vector containing a hypoxia response element isolated from the metallothionein II promoter (e.g., an HRE contained in a fragment having the sequence represented as SEQ ID NO:35), a promoter and a deleterious

gene (e.g., TNF). Exposing the cell to hypoxic conditions enhances expression of the deleterious gene, and expression of the gene is effective to cause injury to the cell.

The invention further includes a substantially isolated polynucleotide having a sequence corresponding to hypoxia response enhancer element(s) (HREE(s)) present in a control region of the muscle pyruvate kinase gene. The element may be derived from the promoter region, 5' untranslated region, or 3' untranslated region. In a related aspect, the invention includes an HRE element derived from a muscle pyruvate kinase gene.

Also included in the invention is a substantially isolated polynucleotide having a sequence corresponding to hypoxia response element(s) present in a control region of the endothelin-1 gene. The element may be derived from the promoter region, 5' untranslated region, or 3' untranslated region. In a related aspect, the invention includes an HRE element derived from an endothelin-1 gene.

Another aspect of the invention includes a substantially isolated polynucleotide having a sequence corresponding to hypoxia response element(s) present in a control region of the enolase 3 (ENO3) gene. The element may be derived from the promoter region, 5' untranslated region, or 3' untranslated region. In a related aspect, the invention includes an HRE element derived from an ENO3 gene. In another related aspect, the invention includes a hypoxia responsive element (HRE) contained in the region of the metallothionein II (MTAII) promoter corresponding to SEQ ID NO:35. In a preferred embodiment, the HRE element consists of a sequence derived from SEQ ID NO:35.

These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B show a schematic diagram of the construction of plasmid pGLHRE (Fig. 1B) from plasmid pGL2PV (Fig. 1A).

Figures 2A, 2B, 2C and 2D show a schematic diagram of the construction of plasmids pGLHSA-150HRE (Fig. 2B), pGL α MHC₈₆-HRE (Fig. 2C), and pGLHCA₁₁₈HRE (Fig. 2D), from plasmid pGLHRE (Fig. 2A).

Figures 3A and 3B show a schematic diagram of the construction of plasmid pGL α MHC_{1,2}HRE (Fig. 3B) from plasmid pGLHRE (Fig. 3A).

Figures 4A and 4B show a schematic diagram of the construction of plasmid pGL α MHC_{1,2}HRE-NOS (Fig. 4B) from plasmid pGL α MHC_{1,2}HRE (Fig. 4A).

Figures 5A and 5B show a schematic diagram of the construction of plasmid p α MHC_{1,2}-HRE-Bcl-2 (Fig. 5B) from plasmid pSFFV-Bcl-2 (Fig. 5A).

Figures 6A, 6B, 6C, 6D and 6E show a schematic diagram of the construction of plasmids pGLPKM₄₀₀ (Fig. 6C), pGLPKM_D (Fig. 6D), and pGLPKM₂₃₅ (Fig. 6E) from plasmid pGL2BV (Fig. 6B) and a fragment of the PKM promoter (Fig. 6A; SEQ ID NO:7).

Figures 7A, 7B and 7C show a schematic diagram of the construction of plasmid pGLET-1₇₀₀ (Fig. 7C) from plasmid pGL2BV (Fig. 7B) and a fragment of the ET-1 promoter (Fig. 7A; SEQ ID NO:8).

10 **BRIEF DESCRIPTION OF THE SEQUENCES**

SEQ ID NO:1 is the sense strand nucleotide sequence of a GATA4 enhancer element (Molkentin, *et al.*, 1984).

SEQ ID NO:2 is the nucleotide sequence of muscle pyruvate kinase (PKM) sense strand primer F.

15 SEQ ID NO:3 is the nucleotide sequence of PKM reverse strand primer R.

SEQ ID NO:4 is the nucleotide sequence of endothelin-1 (Et-1) sense strand primer F.

SEQ ID NO:5 is the nucleotide sequence of Et-1 reverse strand primer R.

SEQ ID NO:6 is the nucleotide sequence of hypoxia response enhancer element 1 (HREE1), derived from the erythropoietin (EPO) gene (Semenza and Wang), and containing
20 4 tandem copies of a hypoxia response enhancer (HRE) sequence and cloning linkers.

SEQ ID NO:7 is the nucleotide sequence of a rat muscle pyruvate kinase (PKM) promoter region (Takenaka, *et al.*).

SEQ ID NO:8 is the nucleotide sequence of a human Et-1 promoter region (Inoue, *et al.*).

25 SEQ ID NO:9 is the nucleotide sequence of a human cardiac actin promoter region (Minty and Kedes).

SEQ ID NO:10 is a nucleotide sequence containing a portion of the rat cardiac α -myosin heavy chain promoter region (Mahdavi, *et al.*; GenBank Accession # K01464).

30 SEQ ID NO:11 is a nucleotide sequence containing a portion of the mouse cardiac α -myosin heavy chain promoter region (Gulick, J., *et al.*; GenBank Accession # M62404).

SEQ ID NO:12 is the nucleotide sequence of a human B-cell leukemia/lymphoma 2 (bcl-2) gene (Tsujimoto, *et al.*; GenBank Accession # M13994).

SEQ ID NO:13 is the predicted amino acid sequence from SEQ ID NO:12.

SEQ ID NO:14 is the nucleotide sequence of a rat nitric oxide synthase (bNOS) gene (Bredt, *et al.*; EMBL Accession # X59949).

SEQ ID NO:15 is the predicted amino acid sequence from SEQ ID NO:14.

SEQ ID NO:16 is the nucleotide sequence of a human bcl-2 fusion gene (Seto, *et al.*;
5 EMBL Accession # X06487).

SEQ ID NO:17 is the predicted amino acid sequence from SEQ ID NO:16.

SEQ ID NO:18 is the nucleotide sequence of a human NOS-1 gene (Fujisawa, *et al.*);
DDBJ Accession # D16408; NCBI Seq ID 506339)

SEQ ID NO:19 is the predicted amino acid sequence from SEQ ID NO:18.

10 SEQ ID NO:20 is the nucleotide sequence of a human NOS-SN gene (Nakane, *et al.*;
GenBank Accession # L02881)

SEQ ID NO:21 is the predicted amino acid sequence from SEQ ID NO:20.

SEQ ID NO:22 is the nucleotide sequence of a 256 base pair (bp) 3' EPO-1 hypoxia
response enhancer element (Semenza and Wang).

15 SEQ ID NO:23 is the nucleotide sequence of a 42 bp 3' EPO-1 hypoxia response
enhancer element (Madan, *et al.*).

SEQ ID NO:24 is the nucleotide sequence of an 86 bp rat α MHC promoter region.

SEQ ID NO:25 is the nucleotide sequence of a mouse catalase gene (Reimer, *et al.*;
GenBank #L25069).

20 SEQ ID NO:26 is the predicted amino acid sequence from SEQ ID NO:25.

SEQ ID NO:27 is the nucleotide sequence of a human manganese superoxide dismutase
(SOD) gene (Clair, *et al.*; EMBL #X59445).

SEQ ID NO:28 is the predicted amino acid sequence from SEQ ID NO:27.

SEQ ID NO:29 is the nucleotide sequence of a human β -enolase (ENO3) gene
25 (Giallongo, *et al.*; EMBL #X56832) between nucleotides -628 to +63.

SEQ ID NO:30 is the predicted amino acid sequence from SEQ ID NO:29.

SEQ ID NO:31 is a consensus sequence of a region present in both the PKM and
ENO3 promoters.

SEQ ID NO:32 is the DNA sequence of the -760 fragment of the human metallo-
30 thionein IIA (hMTAIIa) promoter.

SEQ ID NO:33 is the DNA sequence of the -345 fragment of the hMTAIIa promoter.

SEQ ID NO:34 is the DNA sequence of the -163 fragment of the hMTAIIa promoter.

SEQ ID NO:35 is the DNA sequence of the -90 fragment of the hMTAIIa promoter.

SEQ ID NO:36 is a cDNA sequence encoding human tumor necrosis factor (hTNF; EMBL Accession #X01394; Pennica, *et al.*, Shirai, *et al.*).

SEQ ID NO:37 is the predicted amino acid sequence from SEQ ID NO:36.

5 DETAILED DESCRIPTION OF THE INVENTION

Definitions

"Ischemia" is defined as an insufficient supply of blood to a specific organ or tissue. A consequence of decreased blood supply is an inadequate supply of oxygen to the organ or tissue (hypoxia). Prolonged hypoxia may result in injury to the affected organ or tissue.

10 "Anoxia" refers to a virtually complete absence of oxygen in the organ or tissue, which, if prolonged, may result in death of the organ or tissue.

"Hypoxic condition" is defined as a condition under which a particular organ or tissue receives an inadequate supply of oxygen.

15 "Anoxic condition" refers to a condition under which the supply of oxygen to a particular organ or tissue is cut off.

"Reperfusion" refers to the resumption of blood flow in a tissue following a period of ischemia.

"Ischemic injury" refers to cellular and/or molecular damage to an organ or tissue as a result of a period of ischemia and/or ischemia followed by reperfusion.

20 An "element", when used in the context of nucleic acid constructs, refers to a region of the construct or a nucleic acid fragment having a defined function. For example, a hypoxia response enhancer element is a region of DNA that, when associated with a gene operably linked to a promoter, enhances the transcription of that gene under hypoxic conditions.

25 The term "operably linked", as used herein, denotes a relationship between a regulatory region (typically a promoter element, but may include an enhancer element) and the coding region of a gene, whereby the transcription of the coding region is under the control of the regulatory region.

30 Two nucleic acid elements are said to be "heterologous" if the elements are derived from two different genes, or alternatively, two different species. For example, a hypoxia response enhancer element from a human erythropoietin gene is heterologous to a promoter from a human myosin gene. Similarly, a hypoxia response enhancer element from a human erythropoietin gene, for example, is heterologous to a promoter from a mouse erythropoietin gene.

"Control region" refers to specific sequences at the 5' and 3' ends of eukaryotic genes which may be involved in the control of either transcription or translation. For example, most eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription initiation site. Similarly, most eukaryotic genes have a CXCAAT
5 region (X may be any nucleotide) 70 to 80 bases upstream from the start of transcription. At the 3' end of most eukaryotic genes is an AATAAA sequence, which may be the signal for addition of the polyadenylation tail to the 3' end of the transcribed mRNA.

"Chimeric gene" refers to a polynucleotide containing heterologous DNA sequences, such as promoter and enhancer elements operably linked to a therapeutic gene. For example,
10 a construct containing a human α -myosin heavy chain (α -MHC) promoter fragment operably linked to a human bcl-2 gene and containing a human erythropoietin gene hypoxia response element comprises an exemplary chimeric gene.

I. Overview of the Invention

15 The present invention relates to chimeric genes having at least three functional elements: (i) a therapeutic gene, (ii) a tissue-specific promoter, and (iii) a hypoxia response enhancer (HRE) element. The tissue-specific promoter in combination with the HRE element directs expression of the therapeutic gene in a selected tissue under hypoxic conditions.

The gene is preferably introduced into a target tissue as part of a complete expression
20 vector in a pharmaceutically-acceptable vehicle, either by direct administration to the target tissue (*e.g.*, injection into the target tissue), or by systemic administration (*e.g.*, intravenous injection). In the latter case, the gene may be targeted to a selected tissue, for example, by incorporating it in a virion expressing a modified envelope protein designed to bind to receptors preferentially expressed on cells from the selected, or targeted, tissue. Regardless of the
25 delivery means, expression of the gene in tissues other than the target tissue, and under conditions other than hypoxic or anoxic is preferably minimal.

As described below, a variety of therapeutic genes, promoters, HRE elements and gene delivery means may be employed in the practice of the present invention.

30 II. Tissue Specific Promoters

A promoter, in the context of the present specification, refers to a polynucleotide element capable of regulating the transcription of a gene adjacent and downstream (3') of the promoter. The promoter may contain all of, or only a portion of, the complete 5' regulatory

sequences of the gene from which it is derived. A sequence in the promoter region is typically recognized by RNA polymerase molecules that start RNA synthesis.

A promoter may be functional in a variety of tissue types and in several different species of organisms, or its function may be restricted to a particular species and/or a particular tissue. Further, a promoter may be constitutively active, or it may be selectively activated by certain substances (*e.g.*, a tissue-specific factor), under certain conditions (*e.g.*, hypoxia, or the presence of an enhancer element in the chimeric gene containing the promoter), or during certain developmental stages of the organism (*e.g.*, active in fetus, silent in adult).

Promoters useful in the practice of the present invention are preferably tissue-specific - that is, they are capable of driving transcription of a gene in one tissue while remaining largely "silent" in other tissue types. It will be understood, however, that tissue-specific promoters may have a detectable amount of "background" or "base" activity in those tissues where they are silent. The degree to which a promoter is selectively activated in a target tissue can be expressed as a selectivity ratio (activity in a target tissue/activity in a control tissue). In this regard, a tissue specific promoter useful in the practice of the present invention typically has a selectivity ratio of greater than about 5. Preferably, the selectivity ratio is greater than about 15.

It will be further understood that certain promoters, while not restricted in activity to a single tissue type, may nevertheless show selectivity in that they may be active in one group of tissues, and less active or silent in another group. Such promoters are also termed "tissue specific", and are contemplated for use with the present invention. For example, promoters that are active in a variety of central nervous system (CNS) neurons may be therapeutically useful in protecting against damage due to stroke, which may effect any of a number of different regions of the brain.

Tissue-specific promoters may be derived, for example, from promoter regions of genes that are differentially expressed in different tissues. For example, a variety of promoters have been identified which are suitable for upregulating expression in cardiac tissue. Included are the cardiac α -myosin heavy chain (α MHC) promoter and the cardiac α -actin promoter.

A further desirable characteristic of promoters useful in the present invention is that they possess a relatively low activity in the absence of activated hypoxia-regulated enhancer elements, even in the target tissues. One means of achieving this is to select promoters of genes encoding proteins that have a relatively low turnover rate in adult tissue, such as the actin and α -MHC promoters described herein. Another means is to use "silencer" elements, which suppress the activity of a selected promoter in the absence of hypoxia.

The level of expression of a gene under the control of a particular promoter can be modulated by manipulating the promoter region. For example, different domains within a promoter region may possess different gene-regulatory activities. The roles of these different regions are typically assessed using vector constructs having different variants of the promoter with specific regions deleted (*i.e.*, deletion analysis). Vectors used for such experiments typically contains a reporter gene, which is used to determine the activity of each promoter variant under different conditions. Application of such a deletion analysis enables the identification of promoter sequences containing desirable activities.

This approach may be used to identify, for example, the smallest region capable of conferring tissue specificity, or the smallest region conferring hypoxia sensitivity.

A number of tissue specific promoters, described below, may be particularly advantageous in practicing the present invention. In most instances, these promoters may be isolated as convenient restriction digest fragments suitable for cloning into a selected vector.

Alternatively, promoter fragments may be isolated using the polymerase chain reaction (PCR; Mullis, Mullis, *et al.*). Cloning of amplified fragments may be facilitated by incorporating restriction sites at the 5' ends of the primers.

Promoters suitable for cardiac-specific expression include the promoter from the murine cardiac α -myosin heavy chain gene. The gene contains a 5.5 kbp promoter region which may be obtained as a 5.5 kbp *SacI/SaII* fragment from the murine α MHC gene (Subramaniam, *et al.*, 1991). Reporter gene constructs utilizing this 5.5 kbp α MHC promoter are expressed at relatively high levels selectively in cardiac tissue (whether or not an HREE is present) and, when present in transgenic animals, are regulated in a similar fashion to the endogenous gene (Subramaniam, *et al.*, 1991).

A smaller fragment of the rat α -MHC promoter may be obtained as a 1.2 kbp *EcoRI/HindIII* fragment (Gustafson, *et al.*). As shown in Example 1 and Table 1, below, constructs utilizing the 1.2 kbp rat α MHC promoter are expressed at a low level in the absence of an HREE, and at an intermediate level in the presence of an HREE. These results indicate that the α MHC_{1,2} promoter is an exemplary promoter to target expression of chimeric genes of the present invention to cardiac tissue. Expression of genes under the control of this promoter fragment is very low in cardiac cells under normal oxygenation conditions, but is increased by about a factor of four under hypoxic conditions when the construct contains HREE1. Expression in cells other than cardiac cells is at background levels.

An 86 bp fragment of the rat α MHC promoter, presented herein as SEQ ID NO:24, restricts expression of reporter genes to cardiac and skeletal muscle (*i.e.*, it has lost some tissue

selectivity). Additional cardiac specificity may be conferred to the fragment by ligating (*e.g.*, blunt end ligating) a 36-mer oligonucleotide (SEQ ID NO:1) containing cardiac-specific GATA4 enhancer elements just upstream of base pair -86 (Molkentin, *et al.*, 1984). This promoter fragment also results in low levels of expression in the absence of additional
5 enhancers such as HRE elements. The low level of basal expression induced by the 86 bp fragment, and the ability to upregulate this basal level of expression with a hypoxia response enhancer element are useful properties for a promoter for use with the present invention.

The sequences of exemplary cardiac-specific promoter regions from the rat and mouse α MHC genes are presented herein as SEQ ID NO:10 and SEQ ID NO:11, respectively. Both
10 sequences end just upstream of the ATG initiation codons of their respective genes. Other cardiac-specific promoters include the cardiac α -actin promoter and the myosin light chain-2 (MLC-2) promoter. Constructs described herein utilizing a 118 bp fragment (SEQ ID NO:9) from the human cardiac α -actin (HCA) promoter result in a relatively low level of cardiac-specific expression, which may be increased by the inclusion of an HREE in the expression
15 construct (Example 1, Table 1). The cardiac-specific myosin light chain-2 promoter may be obtained as a 2.1 kbp *KpnI/EcoRI* fragment from the rat cardiac myosin light chain-2 (MLC-2) gene (Franz, *et al.*).

Prostate-specific promoters include the 5'-flanking regions of the human glandular kallikrein-1 (hKLK2) gene and the prostate-specific antigen (hKLK3; PSA) gene (Murtha, *et al.*;
20 *et al.*; Luke, *et al.*). The renin promoter is suitable for directing kidney-specific expression (Fukamizu, *et al.*), while the aldolase-C promoter (Vibert, *et al.*) or the tyrosine hydroxylase promoter (Sasaoka, *et al.*) may be used to direct expression in the brain. Promoters specific for vascular endothelium cells include the Et-1 promoter (Inoue, *et al.*) and vonWillebrand factor (Jahroni and Lynch) promoter.

25 Tumor-specific promoters include the α -fetoprotein (AFP) promoter, contained in a 7.6 kbp fragment of 5'-flanking DNA from the mouse AFP gene (Marci, *et al.*). This promoter normally directs expression of the AFP gene in fetal liver and is transcriptionally silent in adult tissues. However, it can be abnormally reactivated in hepatocellular carcinoma (HCC), conferring tumor-specific expression in adult tissue (Marci, *et al.*).

30 The above promoters are exemplary promoters for use with the present invention. Other promoters suitable for use with the present invention may be selected by one of ordinary skill in the art following the guidance of the present specification.

III. Hypoxia Response Enhancer Elements

Therapeutic genes contained in constructs of the present invention are preferably expressed at low levels, if at all, under conditions of normal oxygenation (minimizing any side effects). Under conditions of hypoxia, however, expression of the genes is increased, affording
5 protection to the target tissue. The elevated expression under hypoxic conditions is conferred by the presence of one or more hypoxia response enhancer (HRE) elements.

HRE elements contain polynucleotide sequences that may be located either upstream (5') or downstream (3') of the promoter and/or therapeutic gene. The HRE element (HREE) is typically a *cis*-acting element, usually about 10-300 bp in length, that acts on a promoter to
10 increase the transcription of a gene under the control of the promoter. Preferably, the promoter and enhancer elements are selected such that expression of a gene regulated by those elements is minimal in the presence of a healthy supply of oxygen, and is upregulated under hypoxic or anoxic conditions.

Hypoxia response enhancer elements are found in association with a number of genes, including the erythropoietin (EPO) gene. Exemplary HRE elements from the EPO gene are
15 presented herein as SEQ ID NO:6, SEQ ID NO:22 and SEQ ID NO:23. The element having the sequence represented as SEQ ID NO:22 results in approximately a five-fold induction of reporter gene expression under hypoxic conditions (Semenza and Wang), while, the element having the sequence represented as SEQ ID NO:23 results in approximately a 17-fold increase
20 in activity under hypoxic conditions (Madan, *et al.*)

Experiments performed in support of the present invention (*e.g.*, Example 1) demonstrate that expression of constructs containing HREE1 (SEQ ID NO:6) is increased by approximately 5- to 7-fold in response to hypoxic conditions. These results indicate that the HREE1 element is fully functional when fused to muscle and cardiac specific promoters and
25 that muscle and cardiac cells are fully responsive to hypoxia in terms of the regulation of these promoters.

Expression of constructs containing a fragment (SEQ ID NO:29) from the control region of the enolase 3 (ENO3) gene was induced approximately 5 to 8 fold by hypoxia in C2C12 cells and cardiac myocytes respectively (see Table 1). These results suggest that the
30 HREE in the ENO3 promoter fragment may be a particularly effective HREE for hypoxia induction in constructs containing a tissue-specific promoter, such as a cardiac or skeletal muscle promoter.

According to the present invention, exemplary hypoxia response enhancer elements may also be isolated from regulatory regions of both the muscle glycolytic enzyme pyruvate kinase

(PKM) gene (Takenaka, *et al.*), the human muscle-specific β -enolase gene (ENO3; Peshavaria and Day), and the endothelin-1 (ET-1) gene (Inoue, *et al.*). The HRE regions from the PKM gene and the ET-1 gene, identified in experiments performed in support of the present invention (see Materials and Methods, Examples 4 and 5), are presented herein as SEQ ID NO:7 and
5 SEQ ID NO:8, respectively.

Example 4 demonstrates that the expression of pGLPKM, a plasmid containing the HRE element from the PKM gene, in transfected C2C12 myotubes and neonatal cardiac myocytes was increased by 6 ± 2 ($n = 4$) fold in both cell types by incubation of the cells in a hypoxic atmosphere. A portion of this HRE element, obtained by digesting with *Sma*I to cut
10 at an internal *Sma*I site, localized the hypoxia response sequence to a 200 bp fragment. This fragment, termed HREPKM₂₈₅, confers hypoxia-induced expression in C2C12 myotubes and cardiac myocytes that is at least equivalent to that obtained using HREE1 (SEQ ID NO:6).

Both PKM and ENO3 promoters contain a common sequence element (SEQ ID NO:31) located at 5' -88 and -70 bp respectively from the transcription start sites. An oligonucleotide
15 containing this sequence may be sufficient to confer hypoxia response characteristics to constructs of the present invention.

Data presented in Example 5 show that expression of pGLET-1₇₀₀, containing 700 bp of the human ET-1 gene promoter (SEQ ID NO:8), in transfected human arterial endothelial cells was increased approximately 5 -fold by incubation of the cells in a hypoxic atmosphere.
20 No hypoxia-induced increase in pGLET-1₇₀₀ expression was seen in other cell types, including HeLa cells, C2C12 cells, and cardiac myocytes. Accordingly, the 700 bp fragment may be used to target hypoxia regulated genes specifically to cells of the vascular endothelium, since the fragment contains element(s) conferring tissue specificity (i.e., elements effective to target expression exclusively to the vascular endothelium), as well as HRE element(s) effective to
25 upregulate transcription of a gene under control of the fragment during hypoxic conditions.

Data presented in Example 6 show that hypoxic stress can increase transcription from constructs containing fragments of the hMTIIa proximal promoter. Enhancements in CAT activity relative to the aerobic controls were observed at both 8 and 14 hr of hypoxia. The levels of induction (2-3 fold) were within the same range as those found in the cadmium
30 chloride-treated positive controls. Hypoxia responsiveness of the -760 construct (SEQ ID NO:32) was similar to that of the -345 (SEQ ID NO:33) construct.

Deletion analyses described in Example 7 show that extracts from cells transfected with constructs containing the -163 fragment (SEQ ID NO:34) and the -90 fragment (SEQ ID NO:35) showed significant upregulation of reporter activity (luciferase activity) under hypoxic

conditions, with levels of induction (approximately 3.0-fold) similar to those observed in Example 6. These results suggest that at least one HRE element is contained in the proximal 90 bp fragment (SEQ ID NO:35) of the hMTIIa promoter. Such an HRE element may be utilized in the methods and constructs of the present invention.

5 It will be appreciated that deletion analyses such as described in Example 7 may be used to identify the shortest sequence present in the -90 fragment (SEQ ID NO:35) that still confers hypoxia sensitivity or inducibility, and that this shorter sequence may be used as the HRE element in the compositions and methods of the present invention.

10 It will further be appreciated that the present invention includes the use of HRE elements not explicitly identified above. Additional HRE elements may be identified, for example, as detailed in Examples 4 and 5. Further, promoter deletion and mutation analyses (e.g., as described above and in Webster and Kedes) may be used to identify such elements in other hypoxia responsive genes. A number of such responsive target genes have been shown to be induced when cells are exposed to hypoxia *in vitro* (e.g., Heacock and Sutherland).

15 It will also be appreciated that, in certain circumstances, the tissue-specific promoter and the hypoxia response enhancer element(s) of the present invention may be derived from a contiguous polynucleotide sequence from a single gene (e.g., as shown above for the ET-1 promoter region, which contains HRE element(s) and also imparts endothelial cell-specific expression).

20

IV. Therapeutic Genes

The present invention may be used to alleviate a number of disease conditions resulting from hypoxic and/or anoxic conditions due to ischemia where cell and tissue damage results from ischemia and ischemia followed by reperfusion. The invention is particularly suitable in cases where the subject is diagnosed to be at risk for an ischemic episode in a particular tissue.

25 For example, it is recognized that virtually all surviving heart attack victims are at significantly increased risk for recurrent episodes of myocardial ischemia. Such subjects would benefit from the introduction of constructs capable of expressing therapeutic genes into their cardiac tissue in order to decrease the risk of injury to the tissue during any subsequent ischemic episodes. Such constructs may serve to protect, for example, cardiac and vascular endothelial tissues from ischemic damage and thereby prevent the progression of the heart disease.

30 Recurrent ischemia and reperfusion typically results in oxidative damage to cells from reactive oxygen species (free radicals), such as peroxides, that are generated during redox

switching (Frei). Contact of fresh blood with damaged or dead cells induces the influx of neutrophils, or pus cells, which kill heart cells which would otherwise have recovered. Much of the damage caused by neutrophils has been attributed to superoxide ions. The superoxide anion can damage tissue in several ways. The interaction of the superoxide anion with hydrogen peroxide leads to the production of hydroxyl radicals which are potentially toxic and react rapidly with most organic molecules. Lipids, proteins, and nucleic acids may all be primary targets for such oxidative damage. The extent and type of damage depend on the severity and nature of the hypoxic stress. For example, the stress may cause cellular damage, initiating an inflammatory response with neutrophil attack and subsequent tissue necrosis. Alternatively, the stress may initiate apoptosis (programmed cell death) to eliminate the damaged cells.

Regardless of the mechanism by which tissue death occurs (necrosis or apoptosis), the damage caused by ischemia-reperfusion episodes is typically the result of redox reactions and is quantitatively related to the severity and duration of the ischemia. For example, in the case of the myocardium, a severe heart attack may result in extensive damage (*e.g.*, infarction of 30% to 40% of the left ventricle), whereas moderate angina and silent repetitive ischemia may result in relatively minor damage during each episode.

While the pathology of ischemia in tissues is complex, resulting in multiple potential targets for therapeutic intervention, several classes of targets are particularly suitable for therapeutic intervention in accordance with the teachings of the present invention. These include anti-oxidant systems, that may intervene immediately at the sites of intracellular redox reactions to minimize damage, and vasodilator systems, that may minimize the severity of the ischemia by increasing blood flow to vulnerable tissues. Antioxidant proteins amenable for use with the present invention include gene products of Bcl-2, catalase and superoxide dismutase (SOD) genes, while proteins with vasodilative properties include nitric oxide synthase (NOS), which produces the vasodilator nitric oxide (NO).

Bcl-2, an integral inner mitochondrial membrane protein of relative molecular mass ~ 25 kDa, has been shown to protect certain cells against apoptosis (Hockenbery, *et al.*, 1990) by acting as an antioxidant (Hockenbery, *et al.*, 1993). Bcl-2 may be an effective therapeutic gene for reducing damage to tissues during ischemic episodes because apoptosis may be a common response of many tissues, including the heart, to oxidative stress (Williams and Smith; Gottlieb, *et al.*

The enzyme superoxide dismutase (SOD) catalyzes the decomposition of the superoxide anion to peroxide. Enzymes such as superoxide dismutase, free radical scavengers or agents

which prevent the influx on neutrophils are able to increase the salvage of heart muscle cells. The enzyme catalase in turn catalyzes the conversion of peroxides to water. Exemplary sequences of a SOD gene and a catalase gene are presented herein as SEQ ID NO:27 and SEQ ID NO:25, respectively. The sequence presented herein as SEQ ID NO:27 encodes a manganese SOD, which has a relatively long half-life. A related sequence, of a human Cu/Zn SOD, may be found in Gorechi, *et al.* The Cu/Zn SOD has a shorter half-life than the manganese SOD.

Endothelial-derived nitric oxide (NO) regulates the expression of vasoconstrictors and growth factors by the vascular endothelium (Kourembanas, *et al.*). Under hypoxia, endothelial cells typically increase expression and secretion of endothelin-1 (ET-1), a potent vasoconstrictor. This increase in expression can be reduced or prevented by exposure to NO (Kourembanas, *et al.*). One of the effects of ET-1 induced vasoconstriction is decreased blood flow to the affected organ or tissue, which can exasperate hypoxic damage due to ischemia. According to the present invention, such damage may be reduced by providing NO to the affected tissue through the expression of a NOS gene under the control of a vascular epithelium or cardiac-specific promoter and hypoxia response enhancer element.

Therapeutic genes of the present invention may be preferably derived from the same or related species as the one to which the methods and compositions of the present invention are applied. For example, for therapeutic treatment of a dog, it may be desirable to utilize a construct containing a therapeutic gene cloned from a dog. Similarly, for treatment of human conditions, it may be desirable to utilize therapeutic genes cloned from human-derived nucleic acids.

The genes encoding the proteins discussed above represent exemplary therapeutic genes useful in the practice of the present invention. It will be appreciated, however, that following the teachings and guidance of the present specification, one of skill in the art may select other therapeutic genes effective to reduce cellular damage due to hypoxia or ischemia, and that the use of such genes is considered to be within the scope of the present invention.

V. Deleterious Genes

In another aspect, the present invention includes constructs containing deleterious genes, rather than therapeutic genes. Expression of the deleterious genes is targeted to tissues which are harmful (*e.g.*, malignant tumors) or otherwise undesirable. Promoters and hypoxia response elements may be selected as described above. Promoters useful in this aspect of the invention preferably restrict expression only to the undesirable tissue. For example, as

discussed above, the AFP promoter can be activated in hepatocellular carcinoma (HCC), conferring tumor-specific expression in adult tissues (Marci, *et al.*).

Deleterious genes include a viral thymidine kinase gene (tk), such as the herpes simplex virus (HSV) tk. This gene is not deleterious by itself, but when expressed, viral TK can phosphorylate ganciclovir (GCV), turning GCV into a cytotoxic compound. Since tumor cells are typically hypoxic, constructs having a tumor-specific promoter operably linked to a viral tk and an HREE may be used in conjunction with GCV to selectively kill tumor cells. Another exemplary deleterious gene is tumor necrosis factor (TNF). TNF is a growth factor that rapidly and induces programmed cell death or apoptosis (Cleveland and Ihle, 1995).

10

VI. Expression Vectors

Chimeric genes of the present invention are preferably incorporated into expression vectors capable of expressing a therapeutic gene product in a selected eukaryotic host cell (*i.e.*, a target tissue). Such expression vectors may contain, in addition to the chimeric gene, various other sequences useful for effective expression of the therapeutic gene in selected tissues. Such sequences may include, for example, sequences necessary for the termination of transcription. These sequences are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding the desired therapeutic protein. The 3' untranslated regions may also include transcription termination sites.

Molecular techniques and methods useful in the construction of expression vectors are well known in the art (*e.g.*, Ausubel, *et al.*, Sambrook, *et al.*). Vector constructs made in support of the present invention are designed to express either a reporter gene (*e.g.*, luciferase), or therapeutic genes (*e.g.*, Bcl-2 or NOS). Therapeutic gene expression is under the control of either a ubiquitous promoter (*e.g.*, SV40), or a tissue-specific promoter (*e.g.*, striated muscle or cardiac-specific promoter). Further regulation of expression by hypoxia or anoxia is provided by inclusion of hypoxia response enhancer (HRE) elements (*e.g.*, from the erythropoietin (EPO) gene, muscle specific pyruvate kinase (PKM) gene, enolase 3 (ENO3) gene or the endothelial cell endothelin-1 (Et-1) gene).

The generation of exemplary constructs is described in the Materials and Methods section, below. The results of *in vitro* experiments to assess the performance of constructs having HREE1 and tissue specific promoters are presented in Example 1 and Table 1. The relative amount of gene expression was measured using a reporter gene (luciferase) in place of a therapeutic gene.

The data shown in Table 1 demonstrate that cells containing constructs having a hypoxia response enhancer element, such as HREE1, in combination with a compatible promoter, express the reporter at levels that are 5 to 7 times greater under hypoxic conditions than under aerobic conditions, and that HREE1 is equally active in different cells and independent of the promoter. The data also demonstrate that expression of constructs containing α -MHC promoters is cardiac specific, and that the basal (aerobic) expression from α -MHC_{1,2} and HCA promoters is relatively low. Further, the data indicate that muscle and cardiac cells are fully responsive to hypoxia in terms of the regulation of these promoters.

In vivo experiments conducted with plasmids pGLHRE and pGLHCA₁₁₈HRE (Example 2, Table 2) demonstrate that gene expression in hearts of rats injected with the plasmids and subjected to ischemia was approximately 2-fold higher than expression in hearts from control animals (not subjected to ischemia). These results indicate that the direct injection of therapeutic constructs of the present invention into cardiac tissue *in vivo* is effective to result in the expression of genes carried on those plasmids. Further, these results indicate that expression vectors carrying chimeric genes of the present invention are effective to result in significantly increased levels of expression in response to hypoxia caused by ischemia *in vivo*.

Since expression was measured at 20 hours after a brief (20 minute) episode of ischemia, it will be appreciated that (i) hypoxia-induced expression may peak significantly earlier than 20 hours, and (ii) repeat ischemic episodes may upregulate expression more the single experimental episode used herein. Accordingly, the 2-fold induction may be an underestimate of the level of enhancement of transcription/expression caused by ischemia.

While the experiments described above were performed with cardiac tissue, it will be appreciated that one of ordinary skill in the art having the benefit of the present specification may perform similar manipulations with other tissues subject to ischemic and or ischemic/reperfusion injury, and that such procedures are within the scope of the present invention.

In vitro experiments (Example 3) demonstrate that cells transfected with reporter (pGLHRE, pGLHCA₁₁₈HRE, pGL α MHC_{1,2}HRE) and therapeutic (pSFFV-Bcl-2 and pNOS-HRE) constructs appear normal and respond to stimuli as expected. Reporter-transfected cells differentiate normally and respond to hypoxia with the predicted induction of reporter, while NOS and bcl-2-transfected cells appear normal both during the hypoxia and during subsequent reoxygenation. These results suggest that inclusion of HRE elements, Bcl-2 over-expression, and hypoxia-induced over-expression of NOS is not toxic or deleterious to muscle cells *in vitro*.

These results also suggest that expression vectors carrying therapeutic genes of the present invention may be effective to protect tissues from ischemic damage. Such protective effects may be assayed in an animal model by, for example, infecting myocardial tissue with an expression vector containing a chimeric gene of the present invention, such as an adenoviral
5 vector expressing a therapeutic gene (*e.g.*, Bcl-2 or SOD), a cardiac-specific promoter, and an HRE element, as described, for instance, in Example 2.

Following infection, the animals may be subjected to repeat ischemic episodes (*e.g.*, 30 minutes to 1 hour) followed by reperfusion (*e.g.*, 1 to 8 hours). Following the last reperfusion, the animals may be sacrificed and the ischemic regions of the myocardium may
10 be tested for the presence and extent of infarction as described, for example, by Thornton, *et al.*, and for the presence of apoptosis as described, for example, in Gottlieb, *et al.* Sample biopsies may also be assayed for expression of the therapeutic gene by Northern blots.

Similar experiments may be performed using constructs directed (*e.g.*, via an appropriate promoter) to other tissues, such as brain, kidney and vascular endothelium.

15 Examples 8 and 9 describe exemplary constructs containing an HRE element from the hMTIIa promoter and a deleterious gene (TNF). The examples describe the testing of such constructs both *in vitro* (Example 8) and *in vivo* (Example 9).

VII. Delivery of Constructs to Cells and Tissues

20 Any of a variety of methods known to those skilled in the art may be used to introduce chimeric genes of the present invention into selected target tissue cells. For example, gene therapy of cardiac tissue has included lipofection, retrovirus and adenovirus-mediated gene transfer, and injection of naked DNA directly into the vascular endothelium or cardiac tissue (Nabel, *et al.*; Lin, *et al.*; Leclerc, *et al.*; Flugelman, *et al.*). These and other methods are
25 discussed more fully in the sections below.

Viral-Mediated Gene Transfer.

Host cells may be transfected with chimeric genes of the present invention by infection with mature virions containing hybrid vectors (the chimeric genes along with selected viral
30 sequences). The virions used to transfect host cells are preferably replication-defective, such that the virus is not able to replicate in the host cells.

The virions may be produced by co-infection of cultured host cells with a helper virus. Following coinfection, the virions are isolated (*e.g.*, by cesium chloride centrifugation) and any remaining helper virus is inactivated (*e.g.*, by heating). The resulting mature virions contain

a chimeric gene of the present invention and may be used to infect host cells in the absence of helper virus. Alternatively, high titers of replication-defective recombinant virus, free of helper virus, may be produced in packaging cell lines containing those components for which the virus is defective (Miller).

5 Several types of viruses, including retroviruses, adeno-associated virus (AAV), herpes virus, vaccinia virus, and several RNA viruses may be amenable for use as vectors with chimeric gene constructs of the present invention. Each type of virus has specific advantages and disadvantages, which are appreciated by those of skill in the art. Methods for manipulating viral vectors are also known in the art (*e.g.*, Grunhaus and Horowitz; Hertz and Gerard; and
10 Rosenfeld, *et al.*)

Retroviruses, like adeno-associated viruses, stably integrate their DNA into the chromosomal DNA of the target cell. Unlike adeno-associated viruses, however, retroviruses typically require replication of the target cells in order for proviral integration to occur. Accordingly, successful gene transfer with retroviral vectors depends on the ability to at least
15 transiently induce proliferation of the target cells.

Retroviral vectors are attractive in part due to the efficiency of transfection -- some vectors can stably transduce close to 100% of target cells. The use of retroviral vectors for *in vivo* gene therapy has been limited, in part, by the requirement of appropriate viral receptors on the target cell. Because the identities of most retroviral receptors are unknown, it has not
20 been possible to determine the distribution of receptors in different cell types. Accordingly, the targeting of specific cell types by retroviral vectors has in many cases proven problematic.

This difficulty may be circumvented by modifying the envelope protein of the retrovirus to contain a ligand for a known endogenous (not necessarily viral) receptor expressed on the target cells. An application of this technique is described in detail by Kasahara. Preferably,
25 the virus also contains an unmodified envelope protein to facilitate cell entry. A number of receptors, such as desmin, E-selectin, and A-CAM, are expressed preferentially on cardiac cells and may be amenable to this approach (*e.g.*, Hansen and Stawaski; Lefer, *et al.*; Youker, *et al.*).

Adeno-associated viruses are capable of efficiently infecting nondividing cells and
30 expressing large amounts of gene product. Furthermore, the virus particle is relatively stable and amenable to purification and concentration. Replication-defective adenoviruses lacking portions of the E1 region of the viral genome may be propagated by growth in cells engineered to express the E1 genes (Jones and Shenk; Berkner; Graham and Prevea). Most of the currently-used adenovirus vectors carry deletions in the E1A-E1B and E3 regions of the viral

genome. A number of preclinical studies using adenoviral vectors have demonstrated that the vectors are efficient at transforming significant fractions of cells *in vivo*, and that vector-mediated gene expression can persist for significant periods of time (Rosenfeld, *et al.*; Quantin, *et al.*; Stratford-Perricaudet, *et al.*, 1992a; Rosenfeld, *et al.*; L. D. Stratford-Perricaudet, *et al.*, 1992b; Jaffe, *et al.*). Several studies describe the effectiveness of adenovirus-mediated gene transfer to cardiac myocytes (Kass-Eisler, *et al.*; Kirshenbaum, *et al.*).

Herpes virus vectors (Breakefield and DeLuca; Freese, *et al.*) are particularly well suited for the delivery and expression of foreign DNA in cells of the central nervous system (CNS), since they can efficiently infect mature, postmitotic neurons. Methods for manipulating the vectors and transfecting CNS cells are well known (see, *e.g.*, Kennedy and Steiner; Yung). A number of studies describe methods for transplanting genetically modified cells into different regions of the brain (Malim, *et al.*; Rossi and Sarver; Sullenger, *et al.*; Morgan, *et al.*; Chatterjee, *et al.*; Malin, *et al.*; Hope, *et al.*). Studies utilizing direct injection of vectors into CNS tissue have also been performed (*e.g.*, Zhang, *et al.*).

Naked DNA injection

Plasmids bearing chimeric genes of the present invention may be purified and injected directly into a target tissue, as exemplified in Example 2 for rat cardiac tissue. The data discussed in Example 2 demonstrate that cardiac injection of plasmid suspended in saline buffer is effective to result in expression of the plasmid in the cardiac cells. Similar approaches have been used successfully by others to express, for example, exogenous genes in rodent cardiac and skeletal muscle (Wolf, *et al.*; Ascadi, *et al.*, 1991a; Ascadi, *et al.*, 1991b; Lin, *et al.*; Kitsis, *et al.*).

Liposome-Mediated Gene Transfer

Liposomes may be employed to deliver genes to target tissues using methods known in the art. The liposomes may be constructed to contain a targeting moiety or ligand, such as an antigen, an antibody, or a virus on their surface to facilitate delivery to the appropriate tissue. For example, liposomes prepared with ultraviolet (UV) inactivated Hemagglutinating Virus of Japan (HVJ) may be used to deliver DNA to selected tissues (Morishita, *et al.*).

The liposomes may also be surface-coated, *e.g.*, by incorporation of phospholipid - polyethyleneglycol conjugates, to extend blood circulation time and allow for greater targeting via the bloodstream. Liposomes of this type are well known.

Receptor-Mediated Gene Transfer

Receptor-mediated endocytic pathways for the uptake of DNA may permit the targeted delivery of genes to specific cell types *in vivo*. Receptor-mediated methods of gene transfer involve the generation of complexes between plasmid DNA and specific polypeptide ligands (Wu) that can be recognized by receptors on the cell surface. One of the problems with receptor-mediated uptake for gene delivery is that the endocytic vesicles formed during this process may be transported to the lysosome, where the contents of the endosome are degraded. Methods have been developed to facilitate escape of the DNA from the endosome during the course of its transport. For example, either whole adenovirus (Wagner, *et al.*, 1992a; Christiano, *et al.*) or fusogenic peptides of the influenza HA gene product (Wagner, *et al.*, 1992b) may be used to induce efficient disruption of DNA-containing endosomes.

Administration of Constructs

In cases such as those outlined above, where a vector may be targeted to selectively transfect a specific population of cells, it will be understood that in addition to local administration (such as may be achieved by injection into the target tissue), the vector may be administered systemically (e.g., intravenously) in a biologically-compatible solution or pharmaceutically acceptable delivery vehicle. Vector constructs administered in this way may selectively infect the target tissue. According to the present invention, the presence of a target tissue-specific promoter on the construct provides an independent means of restricting expression of the therapeutic gene.

VIII. Applications

A. Therapeutic Applications

Compositions and methods of the present invention may be useful to prevent tissue damage and/or death, due to ischemia and/or subsequent reperfusion, in a variety of tissues. As stated above, an exemplary application is in the reduction of damage due to recurrent myocardial ischemia following a heart attack. The expression of therapeutic genes in the cardiac tissue of heart attack victims may decrease the risk of injury to the tissue during any subsequent ischemic episodes.

Similarly, subjects who have been diagnosed with transient cerebral ischemia, blood clots or other risk factors for stroke may benefit from the use of hypoxia-inducible brain-specific constructs. Subjects diagnosed with acute or chronic renal failure are at greater risk for further ischemic damage to the kidneys (*e.g.*, Rosenberg and Paller). Such subjects may

benefit from a therapeutic gene under the control of a kidney-specific promoter, expression of which is enhanced by hypoxic conditions. A variety of other tissues diagnosed as "at risk" for ischemia may be similarly protected, as will be appreciated by one of skill in the art having the benefit of the present specification.

- 5 In addition to the utilities discussed above, compositions (*e.g.*, expression vectors containing chimeric genes of the present invention) and methods of the present invention also have a number of applications in animal medicine. Although animals do not usually develop classical atherosclerosis, cardiomyopathies are very common. A number of species develop ischemia-related syndromes, including arteritis, vasculitis, and related vasculopathies, that result
- 10 in direct redox damage to cells and tissues, particularly to vascular walls and myocardial tissues. Such conditions may be alleviated by administration of chimeric genes of the present invention.

- A common and serious condition in horses and ponies involves ascending colonic ischemia, usually caused by strangulation obstruction (Dabareiner, *et al.*; Sullivan, *et al.*;
- 15 Wilson and Stick). A related disease in dogs is called gastric dilation-volvulus (Lantz, *et al.*). Treatment of these disorders typically involves surgical removal of the obstruction. Reperfusion following such surgery can result in significant injury to reperfused tissues, and typically triggers an inflammatory response with progressive tissue necrosis. The reperfusion may also results in death of the animal due to cardiogenic shock. Compositions and methods
- 20 of the present invention may be used therapeutically to treat such conditions, and to provide protection to vulnerable tissues, including heart and vascular endothelium, during the treatment of the above syndromes.

- Another utility of the present invention is the treatment of cardiac disease in cats and dogs (Miller, *et al.*). A variety of forms of cardiovascular disease have been described in both
- 25 cats and dogs, including dilated cardiomyopathy, left ventricular hypertrophy, and hyperthyroidism (Fox, *et al.*; Atkins, *et al.*). Systemic necrotizing vasculitis, a condition that may be analogous to atherosclerosis in humans (with regard to plaque formation and intimal proliferation), has been described in Beagles (Scott-Moncrieff, *et al.*). Each of these conditions may involve ischemia and reperfusion redox injuries to cardiac and vascular tissue that may be
- 30 treated using the methods and compositions of the present invention.

B. Reporter Constructs for Diagnostic Applications

The present invention may also be employed in diagnostic applications, where it is desirable to localize the site of hypoxia or anoxia. According to this aspect of the invention,

therapeutic genes are replaced by reporter genes, such as those used in experiments performed in support of the present invention (*e.g.*, luciferase). The chimeric genes containing the reporter genes under the control of a selected promoter and a hypoxia response element are introduced into a tissue where it is desirable to localize the site of hypoxia. Hypoxia is
5 localized by increased expression of the reporter gene.

The following examples illustrate but in no way are intended to limit the present invention.

10

MATERIALS AND METHODS

Unless indicated otherwise, chemicals and reagents were obtained from Sigma Chemical Company (St. Louis, MO) or Mallinckrodt Specialty Chemicals (Chesterfield, MO), restriction endonucleases were obtained from New England Biolabs (Beverly, MA), and other modifying enzymes and biochemicals were obtained from Pharmacia Biotech (Piscataway, NJ), Boehringer
15 Mannheim (Indianapolis, IN) or Promega Corporation (Madison, WI). Materials for media for cell culture were obtained from Gibco/BRL (Gaithersburg, MD) or DIFCO (Detroit, MI). Unless otherwise indicated, manipulations of cells, bacteria and nucleic acids were performed using standard methods and protocols (*e.g.*, Titus; Sambrook, *et al.*; Ausubel, *et al.*).

20 A. Definitions

"Transformation" means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integration. Several transformation methods are commonly used in the art, and may be found, for example, in Ausubel, *et al.*, and Sambrook, *et al.*

25 "Transfection" refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, CaPO_4 and electroporation. Successful transfection is generally recognized when any indication of the operation of the expression vector occurs within the host cell.

30 "Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. "Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences (restriction sites) in the DNA. The

various restriction enzymes used herein are commercially available (*e.g.*, New England Biolabs, Beverly, MA) and their reaction conditions are known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of a plasmid or of a DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA
5 fragments for plasmid construction, typically 5 to 10 μg of DNA are digested with about 20 to 40 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about one hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion, the reaction products are run on a gel (*e.g.*, agarose) to isolate desired
10 fragments.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (*e.g.*, Sambrook, *et al.*). Unless otherwise noted, ligation may be accomplished using known buffers and conditions with 10 units of T4 DNA ligase per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

15 "Filling" or "blunting" refer to the procedures by which the single stranded end in the cohesive terminus of a restriction enzyme-cleaved nucleic acid is converted to a double strand. This eliminates the cohesive terminus and forms a blunt end. This process is a versatile tool for converting a restriction cut end that may be cohesive with the ends created by only one or a few other restriction enzymes into a terminus compatible with any blunt-cutting restriction
20 endonuclease or other filled cohesive terminus. Typically, blunting is accomplished by incubating 2-15 μg of the target DNA in a buffer containing 10 mM MgCl_2 , 1 Mm dithiothreitol, 50 mM NaCl, 10 mM Tris (pH 7.5) at about 37°C in the presence of 8 units of the Klenow fragment of DNA polymerase I (Boehringer Mannheim, Indianapolis, IN) and 250 μM of each of the four deoxynucleoside triphosphates (Boehringer Mannheim). The incubation
25 is generally terminated after about 30 min. The reaction products may be purified using standard phenol and chloroform extraction methods followed by ethanol precipitation.

"Northern" blotting is a method by which the presence of a cellular mRNA is confirmed by hybridization to a known, labelled oligonucleotide, DNA or RNA fragment. For the purposes herein, unless otherwise provided, Northern analysis shall mean electrophoretic
30 separation of RNA, typically mRNA, on agarose (*e.g.*, 1%) in the presence of a denaturant (*e.g.*, 7% formaldehyde), transfer to nitrocellulose or nylon membrane, hybridization to the labelled fragment, washing, and detection of the labeled fragment, as described by Sambrook, *et al.*

B. Cells and Media

HeLa cells, Hep G2 cells and C2C12 myoblasts were obtained from the American Type Culture Collection (ATCC; Rockville, MD). Human arterial endothelial cells were obtained from Clonetics Corp. (San Diego, CA). Unless otherwise indicated, the cells were grown at 37°C under 5 or 10% CO₂ in MEM or DMEM medium (Gibco/BRL) containing 10% fetal bovine serum (Gibco/BRL).

Cardiac myocytes were isolated and cultured as described previously (Bishopric, *et al.*, Webster and Bishopric, 1992). Briefly, hearts from about 30 (three litters) were minced and subjected to serial trypsin digestion to release single cells. After the final digestion, the cells were washed and preplated for 0.5 h in minimal essential medium (MEM; Gibco/BRL, Gaithersburg, MD) with 5% fetal calf serum (FCS; Gibco/BRL). Nonattached cells were replated in 60-mm Falcon dishes (Becton Dickinson Labware, Lincoln Park, NJ) at a density of about 2.5×10^6 cells per dish in MEM containing 5% fetal calf serum, 2.0 g/l glucose and 10 mM HEPES, and grown at 37°C under 5 or 10% CO₂.

15

C. DNA

1. Therapeutic Genes

Bcl-2 cDNA was obtained in the expression vector pSFFV-Bcl-2 from Dr. Stanley Korsmeyer (Washington University, St. Louis, MO; Hockenbery, *et al.*, 1990). Nitric oxide synthase (bNOS) cDNA was obtained from Dr. Solomon Snyder in the vector pNOS (Johns Hopkins University, Baltimore, MD; Bredt, *et al.*, 1991).

20

2. Promoters

(i) Cardiac-specific

p α MHC_{3.5}CAT, containing 5.5 kilobases (Kb) 5' of the mouse α -myosin heavy chain (α MHC) promoter ligated to the chloramphenicol acetyl transferase (CAT) gene, was obtained from Dr. Jeffrey Robbins (University of Cincinnati, College of Medicine, Cincinnati, Ohio; Subramaniam, *et al.*).

25

p α MHC_{2.0}CAT, containing 2.0 Kb of the rat α MHC promoter ligated to the CAT gene, was obtained from Dr. Thomas Gustafson (University of Maryland, Baltimore, MD; Gustafson, *et al.*).

30

p α MHC₈₆CAT, containing 86 base pairs (bp) of the rat α MHC promoter ligated to the CAT gene, was obtained from Dr. Bruce Markham (Medical College of Wisconsin, Milwaukee, Wisconsin). The construct was made by 5' truncation of p α MHC2.0CAT and

blunt end ligation to the CAT gene. The sequence of the 86 bp promoter fragment is provided herein as SEQ ID NO:24.

pHCA₁₁₈CAT, containing 118 bp of the region 5' of the human cardiac α -actin promoter ligated to the CAT gene, was also obtained from Dr. Larry Kedes (Minty and
5 Kedes).

(i) Skeletal muscle-specific

pHSA-150CAT, containing 150 bp of the human skeletal muscle α -actin promoter ligated to the CAT gene, was obtained from Dr. Larry Kedes (University of Southern
10 California, Los Angeles, CA; Muscat and Kedes).

3. Hypoxia Response Elements

A construct containing four tandem copies of the erythropoietin gene 3' hypoxia inducible enhancer element cloned into the *Bam*HI site of pGEM-4Z (Promega Corp., Madison,
15 WI) was obtained from Dr. Greg Semenza (Johns Hopkins University School of Medicine, Baltimore, MD; Semenza and Wang, 1992). The enhancer element fragment, termed herein as HREE1 (SEQ ID NO:6), was excised from the pGEM vector by cleavage with *Sma*I and *Hinc*II for blunt end subcloning into constructs of the present invention (below).

A construct containing 691 bp (-628 to +63) of the β -enolase (ENO3) gene was
20 obtained from Dr. Charlotte Peterson (Veterans Administration Medical Center, University of Arkansas, Little Rock, Arkansas). A sequence containing this region is presented herein as SEQ ID NO:29.

4. Chimeric Genes and Expression Vectors of the Present Invention

25 The vector pGL2PV (plasmid-gene-light-promoter-vector; Promega Corp., Madison, WI), was used as the base vector for the construction of most of the plasmids described below. pGL2PV is a eukaryotic expression vector containing the SV40 early promoter upstream of the luciferase gene. The vector multiple cloning (MCS) site is just upstream of the SV40 promoter, and is designed for the insertion of DNA fragments containing enhancer sequences.
30 pGL2BV (Promega Corp.) is similar to pGL2PV, but it does not contain an SV40 early promoter.

(i) HREE1/luc Constructs with Different Tissue-Specific Promoters

Plasmid pGLHRE (Figs. 1B, 2A, 3A) was made by blunt-ligating the 240 bp HREE1 fragment (SEQ ID NO:6) into the *SmaI* site of the MCS of pGL2PV (Fig. 1A).

5 Plasmid pGLHSA-150HRE (Fig. 2B) was made by digesting pGLHRE with *HindIII* and *SmaI* to drop out the SV40 promoter and replacing it with a 150 bp *HindIII-SmaI* fragment from pHSA-150CAT containing a fragment of the human skeletal actin (HSA) promoter.

Plasmid pGL α MHC₈₆HRE (Fig. 2C) was made by digesting pGLHRE with *HindIII* and *SmaI* to drop out the SV40 promoter and replacing it with a 120 bp *HindIII-EcoRI* fragment from p α MHC₈₆CAT containing 86 bp (SEQ ID NO:24) of the human α -myosin heavy chain
10 (α -MHC) promoter. The *EcoRI* end of the 120 bp fragment was filled in with DNA polymerase I using standard methods (Sambrook, *et al.*) before blunt end ligation to the vector *SmaI* site.

Plasmid pGL α MHC₈₆-GATA-HRE was made by cloning a 36 bp oligonucleotide (SEQ ID NO:1; described above), containing a duplicated GATA 4 box into the *HindIII* site (filled
15 in with polymerase) of plasmid pGL α MHC₈₆HRE, upstream of the 86 bp promoter fragment.

Plasmid pGLHCA₁₁₈HRE (Fig. 2D) was made by digesting pGLHRE with *HindIII* and *SmaI* to drop out the SV40 promoter and replacing it with a 188 bp *HindIII-EcoRI* fragment from pHCA₁₁₈CAT, containing 118 bp of the human cardiac actin (HCA) promoter plus 70 bp of actin exon 1. The *EcoRI* end of the 188 bp fragment was filled in with DNA polymerase
20 I as above before blunt end ligation to the vector *SmaI* site.

Plasmid pGL α MHC_{1.2}HRE (Fig. 3B) was made by digesting pGLHRE with *HindIII* and *SmaI* to drop out the SV40 promoter and replacing it with a 1.2 kb *HindIII-EcoRI* fragment from p α MHC_{2.0}CAT containing 1.2 kb of the human α -MHC promoter. The *EcoRI* end of the 1.2 kb fragment was filled in as above in prior to cloning.
25

(ii) PKM Promoter/luc Constructs

Plasmid pGLPKM₄₆₀, containing 460 bp of the rat muscle specific pyruvate kinase (PKM) gene promoter and 140 bp of the PKM coding sequence (SEQ ID NO:7), was created using polymerase chain reaction (PCR) as follows. PKM-specific primers containing
30 endonuclease restriction sites near their 5' end were designed based on the nucleotide sequence of the PKM gene (Takenaka, *et al.*, 1989). PKM primer F (SEQ ID NO:2) contained a *KpnI* site, while PKM primer R (SEQ ID NO:3) contained a *XhoI* site. PCR was carried out using the above primers and 1 μ g of rat heart genomic DNA as a template for 25 cycles using standard procedures and a Perkin-Elmer (Norwalk, CT) DNA thermal cycler. The PCR

product (Fig. 6A) was purified by agarose gel electrophoresis, cut with *KpnI* and *XhoI*, and cloned into *KpnI/XhoI* cut pGL2BV (Fig. 6B; Promega Corp., Madison, WI), generating pGLPKM₄₀₀ (Fig. 6C).

5 Plasmid pGLPKM₂₈₅ (Fig. 6E) was generated by digesting pGLPKM₄₀₀ with *SmaI* to drop out the -460 to -285 portion of the promoter, and religating the vector. pGLPKM_D (Fig. 6D) was generated by digesting pGLPKM₄₀₀ with *SmaI* to isolate the -460 to -285 portion of the promoter, and cloning that fragment into pGL2PV (Promega Corp.) that had been cut with *SmaI*.

10 (iii) Et-1 Promoter/luc Constructs

Plasmid pGLET-1₇₀₀ (Fig. 7C), containing 700 bp of the human ET-1 gene promoter (SEQ ID NO:8), was created using PCR to amplify HeLa cell genomic DNA as described above. ET-1 specific primers were designed based on the promoter sequence (Inoue, *et al.*, 1989) of the ET-1 gene. The forward primer (SEQ ID NO:4) contained *PstI* and *KpnI* sites, 15 while the reverse primer (SEQ ID NO:5) contained *HindIII* and *XbaI* sites. The PCR product (Fig. 7A) was purified by gel electrophoresis, cut with *KpnI* and *HindIII*, and cloned into *KpnI/HindIII* cut pGL2BV (Fig. 7B; Promega Corp.).

(iv) ENO3 Promoter/luc Constructs

20 Plasmid pGLENO₆₂₈ was constructed by cloning a blunt ended genomic DNA containing an ENO3 promoter fragment (-628 to +63; SEQ ID NO:29), isolated from a lambda gt10 human genomic library, into the *SmaI* site of pGL2BV.

(v) Therapeutic Gene Constructs

25 Plasmid p α MHC_{1,2}HRE-NOS (Fig. 4B) was made by digesting plasmid pGL α MHC_{1,2}HRE (Fig. 4A) with *HindIII* and *EcoRV* to drop out the luciferase cDNA and replacing it with a *HindIII/XbaI* fragment from pNOS containing a full length NOS cDNA.

Plasmid p α MHC_{1,2}HRE-Bcl-2 (Fig. 5B) was made by digesting pSFFV-Bcl-2 with *SaII*, blunting the vector as described above, removing the SFFV promoter from the linearized vector 30 with an *EcoRI* digest, and replacing the SFFV promoter with a *SmaI/EcoRI* fragment from pGL α MHC_{1,2}HREE containing the 1.2 kb α MHC promoter fragment and the 240 bp HREE1.

(vi) Other Plasmid Constructs

Plasmid p α MHC_{3.3}HRE-CAT was made by inserting the 240 bp HREE1 immediately 5' of the α MHC promoter of p α MHC_{3.3}CAT.

5 (vi) Adenoviral Constructs

Adenoviral constructs are made using standard methods (*e.g.*, Friedman, *et al.*, 1986; Hertz and Gerard, 1993), as follows.

Construct Ad α MHC1.2Bcl2HREE is made by inserting a 3.34 Kb *EcoRI/HindIII* fragment from p α MHC1.2-Bcl-2 (containing 1.2 Kb of the α -MHC promoter, 1.9 Kb Bcl-2 cDNA, and 240 bp HREE1) into pAPLCMV digested with *EcoRI* and *HindIII* to drop out the CMV promoter and CAT gene. pAPLCMV, which may be obtained from Dr. Larry Kedes (University of Southern California, Los Angeles, CA; Kass-Eisler, *et al.*, 1993), is a base replication deficient adenoviral expression vector. The backbone adenoviral vector for recombination, p9M17, may also be obtained from Dr. Larry Kedes.

15 Recombinant pAPLCMV (pAd α MHC1.2bcl-2HRE) and p9M17 are used to co-transfect 293 cells (ATCC) to propagate the adenovirus.

EXAMPLE 1

Tissue Specific Hypoxia Induced Expression *In Vitro*

20 Constructs pGLHRE, pGLHSA-150HRE, p α MHC_{3.3}HRE-CAT, pGL α MHC_{1.2}HRE, pGLHCA₁₁₈HRE and pGL-Eno₆₂₈ were tested for tissue-specific expression and hypoxia inducibility in HeLa cells, Hep G2 cells, differentiated C2C12 muscle myotubes, and cardiac myocytes.

25 A. Buffers and Solutions

HEPES buffered saline (HeBS; 2X solution)

16.4 g NaCl
11.9 g HEPES acid
0.21 g Na₂HPO₄

30 H₂O to 1 liter

Titrate Ph to 7.05 with 5 M NaOH.

PBS Buffer

35 137 mM NaCl
2.7 mM KCl

4.3 mM Na_2HPO_4
1.4 mM KH_2PO_4

Adjust pH to 7.1.

5 Reconstituted Luciferase Assay Reagent (LAR)

	20 mM	Tricine
	1.07 mM	$(\text{MgCO}_3)_4\text{Mg}(\text{OH})_2 \cdot 5\text{H}_2\text{O}$
	2.67 mM	MgSO_4
	0.1 mM	EDTA
10	33.3 mM	DTT
	270 μM	coenzyme A
	470 μM	luciferin
	530 μM	ATP

15 Cell Culture Lysis Reagent (CCLR; 1X Solution)

	25 mM	Tris-phosphate, pH 7.8
	2 mM	DTT
	2 mM	1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid
20	10%	glycerol
	1%	Triton X-100

A. Cell Transfection

HeLa cells, C2C12 myocytes, and cardiac myocytes were transfected with the indicated
25 plasmid DNA by the standard calcium phosphate procedure (Ausubel, *et al.*).

Briefly, 10^5 cells were plated on a 10-cm tissue culture dish and grown for 3 days. The cells were split 1:10 into 10 ml of medium one day before application of plasmid DNA. DNA for transfection was prepared by resuspending an ethanol-precipitated pellet containing 20 μg of the plasmid DNA in 450 μl ddH₂O and adding 50 μl of 2.5 mM CaCl_2 .

30 500 μl of 2X HeBS were added to a 15 ml conical centrifuge tube, and the solution was aerated by bubbling air with a 10 ml pipette attached to an automatic pipettor (Drummond Instruments, Fisher Scientific, Pittsburgh, PA). The DNA/ CaCl_2 solution was added dropwise, and the resultant mixture was vortexed for 5 seconds and then allowed to sit for 20 minutes at room temperature to form precipitate.

35 The precipitate was added to the dishes containing the cells and the dishes were incubated overnight.

The cells were washed twice with 5 ml PBS and fed with 10 ml of complete medium. The cells were then allowed to recover for 24 hours before incubation under an atmosphere of 1.0% O_2 , 5% CO_2 , 94% N_2 for an additional 20 hours.

B. Exposure to Hypoxic Conditions

Two to three days after transfection, the cells were exposed to atmospheric oxygen (approximately 21% O₂, 5% CO₂, balance N₂; pO₂ = ~160 mmHg), or to hypoxic conditions (approximately 0.5-2.0% O₂, 5% CO₂, balance N₂; pO₂ = ~4-8 mmHg) in an environmental
5 chamber (Anaerobic Systems, San Jose, CA, USA) which was equipped with a Nikon TMS microscope and a continuous readout oxygen electrode (Controls Katharobic, Philadelphia, PA, USA). Unless otherwise indicated, the cells were kept in the chambers for one day prior to assaying for luciferase expression.

10 C. Luciferase Expression

Cells transfected and treated as above were assayed for expression of the luciferase enzyme using a standard reaction protocol (Titus). Briefly, 1 ml of CCLR and 1 ml of LAR were allowed to equilibrate at room temperature. The culture medium in the dish containing the cells to be assayed was removed and the cells were rinsed twice in PBS buffer.

15 Approximately 300 µl of the room-temperature CCLR was added to the dish containing the cells, and the dish was incubated at room temperature for 10-15 minutes. The cells were then scraped off the bottom of the culture dish, and the solution containing the cells was transferred to a micro-centrifuge tube. The tube was centrifuged in a table-top microcentrifuge briefly (about 5 seconds) to pellet large debris.

20 20 µl of the supernatant (cell extract) were mixed with 100 µl of LAR at room temperature, and the light produced was measured for a period of 5 minutes, starting approximately 5 seconds after mixing, with a model #1250 LKB luminometer (BioOrbit, Gaithersburg, MD).

25 D. Results

Data from HeLa, C2C12, and cardiac cells are given in Table 1, below. Values, presented in arbitrary units, represent averages of three or more experiments for each condition.

Table 1

REGULATED EXPRESSION OF UBIQUITOUS- MUSCLE-
AND CARDIAC-SPECIFIC PROMOTERS BY HYPOXIA

	GL2PV		GLHRE		GLHSA ₁₅₀ HRE		α MHC _{1,2} HRE		GLHCA ₁₁₈ HRE		GLENO ₄₃₈	
	A	Hx	A	Hx	A	Hx	A	Hx	A	Hx	A	Hx
HeLa	18	27	56	387	BG		BG		BG		--	
C2C12	189	204	350	1680	46	278	BG		48	248	320	1560
Cardiac	24	27	22	165	18	94	21	85	38	263	210	1610

BG - Background

Data shown in the table demonstrate that (i) none of the tested constructs carrying tissue-specific promoters are expressed above background in fibroblast-derived HeLa cells under either normal or hypoxic conditions, (ii) cells containing constructs having HREE1 and a compatible promoter (including the SV40 and tissue-specific promoters) express the reporter at levels that are ~5 to ~7 times greater under hypoxic conditions than under aerobic conditions; (iii) the HREE1 element is equally active in different cells and independent of the promoter; (iv) the α -MHC_{1,2} promoter expresses in cardiac, but not in skeletal or fibroblast - derived cells, the HCA₁₁₈ promoter expresses in both cardiac and skeletal muscle cells, but not in fibroblast-derived cells, and the HSA₁₅₀ promoter expresses in both skeletal and cardiac muscle, with stronger expression in skeletal muscle; and (v) basal (aerobic) expression from α -MHC_{1,2} HCA₁₁₈, and HSA₁₅₀ promoters is weak.

These results indicate that the HREE1 element is fully functional when fused to muscle and cardiac specific promoters and that muscle and cardiac cells are fully responsive to hypoxia in terms of the regulation of these promoters, and suggest that the α MHC_{1,2} propoter is an exemplary promoter for moderate levels of cardiac-specific expression.

The data also show that both the HREE present in the ENO3 promoter and HREE1, when present in constructs with the SV40 promoter, result in comparable levels of hypoxia induction in skeletal muscle cells. In cardiac cells, however, constructs containing the ENO3 HREE are expressed at significantly higher levels than those containing HREE1. Further, hypoxia increases the level of expression of the ENO3 HREE containing constructs in cardiac cells by over seven-fold, as compared with less than 5-fold in skeletal muscle cells. Plasmid pGLENO₆₂₈ confers induced expression in C2C12 myotubes and cardiac myocytes that is at least equivalent to four copies of the erythropoietin HRE (HREE1) in these cells. These results suggest that the HREE in the ENO3 promoter fragment may be a particularly effective HREE for hypoxia induction in constructs targeted with a tissue-specific promoter to cardiac or skeletal muscle cells.

EXAMPLE 2

Tissue Specific Hypoxia Induced Expression In Vivo Following Injection of Constructs into Target Animal Tissue

Constructs of the present invention were injected directly into cardiac tissue using techniques described in Buttrick, *et al.*, (1992) and Buttrick, *et al.*, (1993). Briefly, adult female Wistar rats were anesthetized with an intraperitoneal injection of chloral hydrate (0.7 ml/100 g of a 4% solution). Cardiac injections were made directly into the apex of the heart through a lateral thoractomy, after which the heart was replaced in the chest, the rats were briefly hyperventilated, and the incision closed. Fifty microliters of a DNA solution containing 2 $\mu\text{g}/\mu\text{l}$ of either pGLHRE or pGLHCA₁₁₈HRE in 20% sucrose and 2% Evans blue were injected through a 27-gauge needle. Following injection the rats were subjected to a 20 min ischemia by cannulation of the coronary artery as described by Smith, *et al.* (1988).

Hypoxia-inducibility of vector expression was assayed as follows. Hearts were excised approximately 20 hours after the induced ischemia and the ventricles were washed with ice-cold phosphate buffered saline (PBS). The tissue was suspended in 1 ml of ice-cold PBS containing 20% sucrose and homogenized with a Polytron (Kinematica, Switzerland) for 45 sec. After centrifugation at $10,000 \times g$ for 10 min supernatants were analyzed for luciferase expression by the assay method described above. Protein was measured using a BioRad assay kit (BioRad Laboratories, Hercules, CA).

The results of the experiments are shown in Table 2, below. Luciferase expression in hearts from rats injected with pGLHRE or pGLHCA₁₁₈HRE and subjected to ischemia

was approximately 2-fold higher than expression in hearts from control animals injected with saline (n=3).

Table 2

ISCHEMIA INDUCIBLE EXPRESSION OF PGLHRE AND
PGLHCA₁₁₈HRE IN RAT HEART

Plasmid	Luciferase Activity Light Units/mg Protein	
	Aerobic	20 min. Ischemic
pGLHRE	1180	2440
pGLHCA ₁₁₈ HRE	88	127
Control	15	21

Rat hearts were injected with plasmids as described above. A 20 min. ischemia was imposed on one group (3 rats) and the other (1 control) was sham operated. Tissue samples were harvested and assayed for luciferase expression 20 hr. later.

These results indicate that the direct injection of plasmid DNA, made in accordance with the teachings of the present specification, into hearts of living mammals is effective to result in the expression of genes carried on those plasmids. Further, these results indicate that expression vectors carrying chimeric genes of the present invention are effective to result in significantly increased levels of expression in response to hypoxia caused by ischemia *in vivo*.

EXAMPLE 3

Stable Expression of Hypoxia Regulated NOS
and Bcl-2 Genes *In Vitro*

10⁶ C2C12 myoblasts were cotransfected with pSV2Neo (Minty and Kedes) and a test plasmid at a ratio of 1:19 (1 μg pSV2Neo + 19 μg test plasmid) using standard methods (Minty and Kedes, 1986). Test plasmids were pGLHRE, pGLHCA₁₁₈HRE, pGLαMHC_{1,2}HRE, pSFFV-Bcl-2, and pNOS-HRE. Cultures were selected on day 2 following transfection with 400 μg/ml of the neomycin drug G418 (Gibco/BRL). Colonies of cells resistant to G418 appeared after 10 to 14 days. The resistant cells were pooled. Mass cultures were assayed for the expression of luciferase as described above or by Northern blot assay (Webster, *et al.*, 1993) for the expression of Bcl-2 or NOS RNA. Stable lines were positive for expression of the transfected genes.

Mass cultures were subjected to differentiation conditions by transferring them to low mitogen medium (DMEM with 2% horse serum) and were analyzed visually for differentiation into myotubes. There was no apparent difference between transfected and control cells. Approximately 40% of cells were fused into multinucleate myotubes after 24 h in low mitogen medium. All cultures contained approximately 74% myotubes after 48h.

Reporter-transfected cells differentiated normally and respond to hypoxia with the predicted induction of reporter. NOS-transfected cells appeared normal both during the hypoxia and during subsequent reoxygenation. A stable line of C2C12 cells that constitutively over-expresses Bcl-2 (without HREE1) was also constructed as described above, and the cells showed normal growth and differentiation characteristics.

Taken together, the data presented above suggest that inclusion of HRE elements, Bcl-2 over-expression, and hypoxia-induced over-expression of NOS is not toxic to muscle cells *in vitro*. Further, the data indicate that the cells may be protected from the deleterious effects of hypoxia by the expression of therapeutic genes (*e.g.*, NOS).

EXAMPLE 4

Expression of pGLPKM Plasmids under Hypoxic Conditions

Plasmid pGLPKM₄₆₀ was transfected into C2C12 cells and cardiac myocytes and assayed for luciferase activity as described in Example 1. The expression of pGLPKM in both transfected C2C12 myotubes and neonatal cardiac myocytes was increased by 6 ± 2 fold ($n = 4$) in both cell types by incubation of the cells in an atmosphere containing 0.5 % O₂, 5% CO₂, balance N₂ (hypoxic conditions) relative to normal conditions, as described in Example 1.

A portion of this HRE element, obtained by digesting with *Sma*I to cut at an internal *Sma*I site, is also effective as a hypoxia response enhancer element. This fragment, termed HREPKM₂₈₅, confers hypoxia-induced expression in C2C12 myotubes and cardiac myocytes similar to that obtained with pGLPKM₄₆₀. This level of hypoxia induction is at least equivalent to that obtained using HREE1 (SEQ ID NO:6).

These results indicate that the PKM promoter fragment contained in the sequence represented as SEQ ID NO:7 contains an HRE element that is effective at enhancing the expression of chimeric genes containing the element under conditions of hypoxia.

The PKM promoter sequence has no significant homology with the erythropoietin HRE consensus, but does share a consensus sequence (SEQ ID NO:31) with the ENO3 promoter fragment (SEQ ID NO:29). This consensus, located approximately 88 bp

upstream of the transcription start site of PKM and approximately 70 bp upstream of the transcription start site of ENO3, may represent an important element for conferring enhancement of expression in response to hypoxia.

5

EXAMPLE 5

Expression of pGLET-1₇₀₀ Plasmids under Hypoxic Conditions

Plasmid pGLET-1₇₀₀ was transfected into human arterial endothelial cells as described in Example 1. The expression of pGLET-1₇₀₀ in these cells was increased 5 fold by incubation of the cells in a hypoxic atmosphere as described above. No significant
10 induction of pGLET-1₇₀₀ was observed in any other cell types tested, including HeLa, C2C12, and cardiac myocytes. Elements contained within the 700 bp sequence have no significant homology with the erythropoietin HRE consensus.

These results indicate that the 700 bp fragment of the human ET-1 gene promoter corresponding to the sequence represented herein as SEQ ID NO:8 is effective to (i) restrict
15 expression of genes under its control to the vascular endothelium, and (ii) confer hypoxia-inducibility on the expression of those genes. Accordingly, this fragment, in conjunction with a therapeutic or reporter gene, may be used in the methods of the present invention to both target expression to a selected tissue (vascular endothelium), and confer enhancement of expression by hypoxia.

20

EXAMPLE 6

Regulation of the Human Metallothionein IIa (hMTIIa) Promoter by Hypoxia

Three DNA fragments derived from the human MTIIa (hMTIIa) promoter, were tested in chloramphenicol acetyltransferase (CAT) reporter gene assays for hypoxia
25 responsiveness. Fragments containing -760 bp (SEQ ID NO:32) and -345 bp (SEQ ID NO:33) of the promoter (including the first +21 bp downstream of the transcription initiation site) were cloned immediately upstream of the bacterial chloramphenicol acetyl transferase (CAT) gene in the pCAT Basic reporter vector (Promega, Madison, WI, USA), generating vectors pCAT-760 and pCAT-345, respectively. These vectors were in turn
30 used to transfect A431 cells (ATCC Accession # CRL-7907) using the standard calcium phosphate method (Ausubel, *et al.*).

Approximately four days after transfection, the transfected cells were exposed to a selection medium comprised of Dulbecco's modified Eagle's medium (DMEM) supplement-

ed with 10% fetal bovine serum and containing 400 $\mu\text{g/ml}$ G418 to select stable clones (the pCAT Basic vector contains a G417/neomycin resistance gene).

Early passages (1-10) of pooled stable clones were used in hypoxia experiments. Three hours before exposure to hypoxia, the medium bathing the cultures was changed.

- 5 The dishes were placed inside specially designed aluminum chambers submerged in a 37°C water bath and attached to a 5% CO_2/N_2 manifold on a vacuum line (Laderoute, *et al.*, 1992). Oxygen was extracted at 37°C over 1.5 hours by 7 cycles of pumping to a fixed pressure followed by filling with 5% CO_2/N_2 . The final O_2 tension in the gas phase was approximately 0.01% of atmospheric O_2 ($p\text{O}_2 < 0.08$ torr).

- 10 Following incubation at 37°C for the indicated time (up to 14 hours), the chambers were opened under 5% CO_2/N_2 in a humidified anaerobic chamber (Anaerobic Systems, San Jose, CA). Aerobic controls were incubated for an equal time period in 5% CO_2/air at 37°C.

- 15 Total protein for CAT assays was harvested as cell lysates using the Triton X-100 method (Laderoute, *et al.*, 1992) in the humidified anaerobic chamber following 8 or 14 hr of hypoxia. The CAT assays were conducted using standard methods (Ausubel, *et al.*). Briefly, Acyl CoA and ^{14}C -labeled chloramphenicol were added to the cell lysates, and modified derivatives of the chloramphenicol were separated from the starting material using thin-layer chromatography. The CAT activity of the extracts was quantitated using the
20 following formula:

$$\% \text{ acetylated} = \frac{\text{counts in acetylated species}}{\text{counts in acetylated species} + \text{counts in nonacetylated chloramphenicol}}$$

- 25 Table 3, below, presents CAT activity data for the -345 bp fragment. The numbers represent the amount of CAT activity in extracts from transfected cells exposed to hypoxia divided by the CAT activity in extracts from transfected cells under normoxic conditions. The hypoxia-regulated transcriptional activation is compared with that caused by cadmium chloride (10 μM), a known activator of hMTIIa transcription (Karin and Herrlich 1989).
30

Table 3

**Characterization of a Hypoxia-Responsive
Element (HRE) in the Promoter of the
Human Metallothionein IIa Gene**

Time (hours)	Transcriptional Activation
CAT (hypoxia)/CAT air	
8	1.8 ± 0.8^a
14	2.7 ± 0.2^b
CAT Cd/CAT (control)	3.9 ± 1.3^b

^aSample SD; n=4

^bSample SD; n=7

These results indicate that hypoxic stress can increase transcription from the hMTIIa proximal promoter. Enhancements in CAT activity relative to the aerobic controls were observed at both 8 and 14 hr of hypoxia. The levels of induction (2-3 fold) were within the same range as those found in the cadmium chloride-treated positive controls. Hypoxia responsiveness of the 760 bp construct was similar to that of the 345 bp construct.

EXAMPLE 7

Deletion Analysis of hMTIIa Promoter

To further characterize the hMTIIa promoter, mouse C2C12 myoblasts were transiently transfected with PCR-generated nested deletion fragments of the -345 bp responsive fragment. Fragments containing -163 bp (SEQ ID NO:34) and -90 bp (SEQ ID NO:35) of the hMTIIa promoter (including the first +21 bp downstream of the transcription initiation site) were inserted immediately upstream of the luciferase reporter gene of the pGL2 plasmid (Promega, Madison, WI), generating pGL2-163 and pGL2-90, respectively. The plasmids were used to transiently transfect the C2C12 cells as described above.

The transfected cells were subjected to hypoxia treatment and cell extracts were made as described above. Luciferase activity of cell extracts was measured using a standard assay (Ausubel, *et al.*). Briefly, ATP and the substrate luciferin were added to the lysate in a luminometer, and total light output was measured. The amount of light was proportional to the amount of luciferase present in the extracts.

Extracts from both pGL2-163- and pGL2-90-transfected cells showed significant upregulation of luciferase activity under hypoxic conditions, with levels of induction

(approximately 3.0-fold) similar to those observed in Example 6, above. These results suggest that at least one HRE is contained in the proximal 90 bp fragment (SEQ ID NO:35) of the hMTIIa promoter.

5

EXAMPLE 8

Induction of Toxic Genes by hMTIIa HRE *In Vitro*

The luciferase coding sequence in the pGL3-Basic promoter vector (Promega) is excised as a *NcoI/XbaI* fragment and replaced with a double-stranded PCR-generated DNA fragment encoding human tumor necrosis factor (hTNF) (SEQ ID NO:37; Shirai, *et al.*).

10 TNF is a growth factor that rapidly and induces programmed cell death or apoptosis (Cleveland and Ihle, 1995), and is not known to be induced by hypoxic stress. The -90 bp hMTIIa fragment (SEQ ID NO:35) is inserted immediately upstream of the TNF gene, resulting in construct hMTIIa-HRE-TNF.

The construct is used to transfect both C2C12 cells (transient transfection) and A431
15 cells (stable transfection) as described above. Transfected cells are then subjected to either normoxic or hypoxic conditions for periods of time ranging from 8 to 24 hr as described above, and induced cytotoxicity of the TNF protein is evaluated using a standard clonogenic assay (*e.g.*, as described in Kowk and Sutherland, 1989). Briefly, several dilutions, 3 replicates per dilution, are plated for each time point, and the cells are incubated undis-
20 turbed in a humidified 37°C incubator for 10-20 days. Cell colonies are stained with methylene blue and colonies with 30 or more cells are scored. Northern and Western analyses are performed immediately after hypoxic treatment to determine induction of TNF.

EXAMPLE 9

Hypoxia-Mediated TNF induction and Tumor Control in an Animal Xenograft Model

To determine the stage at which tumors develop a substantial hypoxic portion, nude mice (Taconic, Germantown, NY, USA) ranging in age from 4-5 weeks, are injected by subcutaneous (s.c.) unilateral injections of about 5×10^6 exponentially growing un-
transfected A431 cells into the dorsum of the right side. Hypoxic regions are identified
30 using a derivative of 2-nitroimidazole etanidazole, the fluorinated bioreductive compound 2-(2-nitro-1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)acetamide (EF5; obtained from Dr. Cameron Koch, Department of Radiation and Oncology, School of Medicine, University of Pennsylvania; Lord, *et al.*, 1993). Etanidazole forms covalent bonds to cellular macromolecules after bioreduction at low oxygen tensions (Lord, *et al.*, 1993). Monoclonal

antibodies raised against these nonphysiological adducts (Lord, *et al.*, 1993) are employed using standard immunohistochemistry to image hypoxic regions in serial frozen sections (7 μ m) from tumors harvested twice per week.

5 A. Testing Reporter Constructs *In Vivo*

Reporter gene constructs containing the luciferase gene under the control of an HRE from the hMTIIa promoter are made as described above and used to stably transfect A431 cells.

Experiments are conducted using three groups of mice, each group injected as described above with one of three types of cells: 1) untransfected cells, 2) stable transfectants containing the empty pGL2 vector and 3) stable transfectants containing the hMTIIA-HRE-pGL2 construct. Groups 1 and 2 are used as negative controls.

The tumors are allowed to grow to a stage at which they contain a substantial hypoxic portion, determined as described above. The mice are then sacrificed, tumors are removed and cut on a cryostat, and the resulting frozen sections are analyzed for luciferase activity and EF5 staining. The degree of overlap between the luciferase activity and EF5 staining in group 3 mice relates to the potential effectiveness of such an HRE-containing construct in a tumor *in vivo*.

20 B. Testing Toxic Constructs *In Vivo*

These experiments are conducted as described above, except that they employ A431 cells transfected with the hMTIIa-HRE-TNF construct or the empty vector (missing both the hMTIIa-HRE and the TNF cDNA). Frozen sections are scored for apoptosis using the "APOTAG" kit (Oncor, Gaithersburg, MD.). Effectiveness of the construct is measured by increased apoptosis in the hypoxic regions of tumors containing the transfected hMTIIa-HRE-TNF construct as compared with tumors containing the empty vector.

While the invention has been described with reference to specific methods and embodiments, it is appreciated that various modifications and changes may be made without departing from the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: SRI International
- (ii) TITLE OF INVENTION: Tissue Specific Hypoxia Regulated
Therapeutic Constructs
- (iii) NUMBER OF SEQUENCES: 37
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Dehlinger & Associates
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 - (C) CITY: Palo Alto
 - (D) STATE: CA
 - (E) COUNTRY: USA
 - (F) ZIP: 94306
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: PCT/IB95/00996
 - (B) FILING DATE: 13-NOV-1995
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- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/365,486
 - (B) FILING DATE: 23-DEC-1994
- (viii) ATTORNEY/AGENT INFORMATION:
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 - (B) REGISTRATION NUMBER: 38,615
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- (ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: GATA4 Enhancer
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAAAGGGCCG ATGGGCAGAT AGAGGAGAGA CAGGA

35

(2) INFORMATION FOR SEQ ID NO:2:

RECTIFIED SHEET (RULE 91)
ISA/EP

46

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: PKM primer F

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AATTGGTACC CGGGCGAGCG CCGGGAGGGT GGA

33

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 32 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: PKM primer R

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTAACTCGAG GCACTATGGC ATTGGCTCTG GG

32

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 41 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: ET-1 primer F

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TATATCTGCA GGTACCGATA GGGAAAAGAC TGGCATGTGC C

41

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 43 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: ET-1 primer R

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TATATAAGCT TCTAGAGACC CGTTCGCCTG GCGCGCAGAT GCA

43

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 240 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: HREE1 (Hypoxia responsive enhancer element 1)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGGGCCCTAC GTGCTGTCTC ACACAGCCTG TCTGACCTCT CGACCTACCG GCCCGGGATC	60
CCGGCCCTAC GTGCTGTCTC ACACAGCCTG TCTGACCTCT CGACCTACCG GCCCGGGATC	120
CCGGCCCTAC GTGCTGTCTC ACACAGCCTG TCTGACCTCT CGACCTACCG GCCCGGGATC	180
CCGGCCCTAC GTGCTGTCTC ACACAGCCTG TCTGACCTCT CGACCTACCG GCCGATCCCG	240

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 560 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: sequence containing PKM promoter frag.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAGTCACCGG GCGGGGCTGG AGGAATGTCC GGGACCTATA AATCTGGGCA ACGCCCTGGT	60
AGGCCAGGGC AGATGGGGCA CCTGGGCAGA ATTCCAAAAT GGGATTATGT AGCCTCTGAG	120
GTCCTAAAGC AACAGGTGGC GGACCACCCG GGGATCTAGG GGTGGTGGCG GCGGTGGACC	180
CGAGGGCGGG TCCTGCCTCC TCACCACTTC CCCATTGGCC ATCAGAATGA CCCATGCGCA	240
ATTTTGGTTT GCAATGTCCT TCCGCCACGG AAGGTAGTCC CCCTCAAAAG GGCAACCTGC	300
TTGTCCCGCC TACCCTGCGA CTCTCTCAGA AGGTGCGGGT GCCTGTTGAG AGGCGGGGCT	360
CTGCTAGCTC CTGCCCGGAT TGGGCGAGGG GCGGGGCTGC GGAGGGATTG CGGCGGCCCCG	420
CAGCAGTGAT AACCTTGAGG CCCAGTCTGC GCAGCCCCGC ACAGCAGCGA CCCGTCCTAA	480
GTCGACAGAC GTCCTCTTTA GGTATTGCAA CAGGATCTGA AGTACGCCCCG AGGTGAGCGG	540
GGAGAACCTT TGCCATTCTC	560

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 713 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: Sequence containing ET-1 promoter

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GATAGGGAAA AGACTGGCAT GTGCCTAAAC GAGCTCTGAT GTTATTTTAA AGCTCCCTTT	60
CTTGCCAATC CCTCACGGAT CTTTCTCCGA TAGATGCAAA GAACTTCAGC AAAAAAGACC	120
CGCAGGAAGG GGCTTGAAGA GAAAAGTACG TTGATCTGCC AAAATAGTCT GACCCCCAGT	180
AGTGGGCAGT GACGAGGGAG AGCATTCCCT TGTTTGA CTG AGACTAGAAT CGGAGAGACA	240
TAAAAGGAAA ATGAAGCGAG CAACAATTAA AAAAAATTCC CCGCACACAA CAATACAATC	300
TATTTAAACT GTGGCTCATA CTTTTCATAC CAATGGTATG ACTTTTTTTC TGGAGTCCCC	360
TCTTCTGATT CTGAACTCC GGGGCTGGCA GCTTGCAAAG GGGAAAGCGGA CTCCAGCACT	420
GCACGGGCAG GTTTAGCAAA GGTCTCTAAT GGGTATTTTC TTTTCTTAG CCCTGCCCCC	480
GAATTGTCAG ACGGCGGGCG TCTGCTTCTG AAGTTAGCAG TGATTTCCTT TCGGGCCTGG	540
CTTATCTCCG GCTGCACGTT GCCTGTTGGT GACTAATAAC ACAATAACAT TGTCTGGGGC	600
TGGAATAAAG TCGGAGCTGT TTACCCCCAC TCTAATAGGG GTTCAATATA AAAAGCCGGC	660
AGAGAGCTGT CCAAGTCAGA CGCGCCTCTG CATCTGCGCC AGGCGAACGG GTC	713

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 118 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: HCA118 promoter fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGAAGGGGAC CAAATAAGGC AAGGTGGCAG ACCGGGCCCC CCACCCCTGC CCCC GGCTGC 60
TCCA ACTGAC CCTGTCCATC AGCGTTCTAT AAAGCGGCCC TCCTGGAGCC AGCCACCC 118

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1588 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: Rat alpha MHC promoter fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GAATTCTCTT ACTATCAAAG GGAAACTGAG TCATGCACCT GCAAAATGAA TGCCCTCCCT 60
GGACATCATG ACTTTGTCCC TGGGGAGCCA GCACTGTGGA ACTCCAGGTC TGAGAGTAGG 120
AGGCACCCCT CAGCCTGAAG CTGTGCAGAT AGCTAGGGTG TAAAAGAGGG AAGGGGGGAG 180
GCTGGAATGG GAGCTTGTGT GTTCGGAGAC AGGGGACAAA TATTAGGCCC GTAAGAGAAG 240
GTGACCCTTA CCCAGTGTGT TCAACTCAGC CTTTCAGATT AAAAAATAACT AAGGTAAGGG 300
CCATGTGGGT AGGGGAGGTG GTGTGAGACG GTCCTGTCTC TCCTCTATCT GCCCATCGGC 360
CCTTTGGGGA GGAGGAAATG TGCCCAAGGA CTAAAAAAGG CCTGGAGCCA GAGGGGCTAG 420
GGCTAAGCAG ACCTTTCATG GGCAAACCTC AGGGCTGCTG TCCTCCTGTC ACCTCCAGAG 480
CCAAGGGATC AAAGGAGGAG GAGCCAGACA GGAGGGATGG GAGGGAGGGT CCCAGCAGAT 540
GACTCCAAAT TTAGGCAGCA GGCACGCGGA ATGAGCTATA AAGGGGCTGG AGCGCTGAGA 600
GCTGTCAGAC CGAGATTCTT CCATCCCAAG TAAGAAGGAG TTTAGCGTGG GGGCTCTCCA 660
ACCGCACCAG ACCTGTCCCA CCTAGAGGGA AAGTGTCTTC CCTGGAAGTG GGCTCCTCCC 720
ACCGGCCTGG GAAGATTCCT CGGTGGGCAG GATGTTCTAC TGGATGCCCC TTCCCTTCCA 780

CTGCCTCCTC CCTCCCTTGT CTTGATTAAT CTTGGCTCTT AGTGTTTACA AAGATTTGCC	840
CGGTGCTGTC TACTCCATCT GTCTCTACTC TCTCTGCCTT GCCTTCTTGT GTGTTCTCCT	900
TTTCCACGTG TTTCTCACTC CACTGCCTCC CCCCCCCCCT TCATTTTTAT CCTTCCTTTC	960
TTTCTGTGTC AGAATGCTGG GAATCAAACC CAGGGCTTCA TACACGTCAA GTAAGCAATC	1020
TCCCAGTGAG TCAAAGCTTT AATCCTCTGG GTGCTGTCTT ACCGAGCCTC ACTCCCTGTC	1080
TTGTCCTGTT CCGTCCTAGT CAGGATCTCT GGTCCGTCTC TCAGCTTCTG CTACTCCTCT	1140
CCCTGCCTGC TCTTCTCTCC GTCCAGCTGC ACCTCTGTGG CGCTCATTC AGCCGTGGTC	1200
CAAATTCTCT GTGAAAAGAT TAACCGGGTG AGAATGCCCC CAGTTTCCCC TGTAGACAGC	1260
AGATCATGAT TTTCCCCAGA AGCCAGACTT CCAGCGCCCC CCCTCTGCCC AGCAACTTGA	1320
CACTCTTAGC AAACCTCAGC CACCCTTCCC CCACATAGAC CAAGTCTTGC AGAGAGCCTT	1380
CCTTCAGATG ACTTCGAGTT CTTGCAAAGG AAGGAGAACT CTTTGTGGCG GGAAGCAGG	1440
CACTTTACAC GGAGTCTGAC GGGAGGTCAT AGGCTATGGC ATAGCAGAGG CAGGGAGGTG	1500
GTGGAATTGG ACTTCGCGCA GAAGCTAAGC ACACACCAGG AATGACATAT CCCTCCTATC	1560
TCCCCATAA GAGTTTAAGA GTGACAGG	1588

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1679 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: Mouse alpha MHC promoter fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAATTCTCTT ACTATCAAAG GGAAACTGAG TCGTGCACCT GCAAAGTGGG TGCTCTCCCT	60
AGACATCATG ACTTTGTCTC TGGGGAGCCA GCACTGTGGA ACTTCAGGTC TGAGAGAGTA	120
GGAGGCTCCC CTCAGCCTGA AGCTATGCAG ATAGCCAGGG TTGAAAGGGG GAAGGGAGAG	180
CCTGGGATGG GAGCTTGTGT GTTGGAGGCA GGGGACAGAT ATTAAGCCTG GAAGAGAAGG	240
TGACCCTTAC CCAGTTGTTC AACTCACCTT TCAGATTAAA AATAACTGAG GTAAGGGCCT	300
GGGTAGGGGA GGTGGTGTGA GACGCTCCTG TCTCTCCTCT ATCTGCCCAT CGGCCCTTTC	360
GGGAGGAGGA ATGTGCCCAA GGAATAAAAA AAGGCCATGG AGCCAGAGGG GCGAGGGCAA	420
CAGACCTTTC ATGGGCAAAC CTTGGGGCCC TGCTGTCTCT CTGTACCTC CAGAGCCAAG	480
GGATCAAAGG AGGAGGAGCC AGGACAGGAG GGAAGTGGGA GGGAGGGTCC CAGCAGAGGA	540
CTCCAAATTT AGGCAGCAGG CATATGGGAT GGGATATAAA GGGGCTGGAG CACTGAGAGC	600

TGTCAGAGAT TTCTCCAACC CAGGTAAGAG GGAGTTTCGG GTGGGGGCTC TTCACCCACA 660
CCAGACCTCT CCCACCTAG AAGGAAACTG CCTTTCCTGG AAGTGGGGTT CAGGCCGGTC 720
AGAGATCTGA CAGGGTGGCC TTCCACCAGC CTGGGAAGTT CTCAGTGGCA GGAGGTTTCC 780
ACAAGAAACA CTGGATGCCC CTTCCCTTAC GCTGTCTTCT CCATCTTCCT CCTGGGGATG 840
CTCCTCCCCG TCTTGGTTTA TCTTGGCTCT TCGTCTTCAG CAAGATTGTC CCTGTGCTGT 900
CCACTCCATC TTTCTCTACT GTCTCCGTGC CTTGCCTTGC CTTCTTGCCT GTCCCTTCCT 960
TCCACCCATT TCTCACTTCA CCTTTTCTCC CTTTCTCATT TGTATTCATC CTTCTTCCT 1020
TCCTTCCTTC CTTCTTCCT TCCTTCCTTC CTTCTTTCT CCCTTCCTTC CTTCTTCCT 1080
TCCTTCCTTC CTTCTTCCT TCCTGTGTCA GAGTGCTGAG AATCACACCT GGGGTTCCTCA 1140
CCCTTATGTA AACAATCTTC CAGTGAGCCA CAGCTTCAGT GCTGCTGGGT GCTCTCTTAC 1200
CTTCCTCACC CCCTGGCTTG TCCTGTTCCA TCCTGGTCAG GATCTCTAGA TTGGTCTCCC 1260
AGCCTCTGCT ACTCCTCTTC CTGCCTGTTT CTCTCTCTGT CCAGCTGCGC CACTGTGGTG 1320
CCTCGTTCCA GCTGTGGTCC ACATTCTTCA GGATTCTCTG AAAAGTTAAC CAGGTGAGAA 1380
TGTTTCCCCT GTAGACAGCA GATCACGATT CTCCCGGAAG TCAGGCTTCC AGCCCTCTCT 1440
TTCTCTGCCC AGCTGCCCCG CACTCTTAGC AAACCTCAGG CACCCTTACC CCACATAGAC 1500
CTCTGACAGA GAAGCAGGCA CTTTACATGG AGTCCTGGTG GGAGAGCCAT AGGCTACGGT 1560
GTAAAAGAGG CAGGGAAGTG GTGGTGTAGG AAAGTCAGGA CTTACATAG AAGCCTAGCC 1620
CACACCAGAA ATGACAGACA GATCCCTCCT ATCTCCCCCA TAAGAGTTTG AGTGACAGA 1679

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5057 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: rat bNOS cDNA
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 349..4638

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ACGTCTGACA AGCTGGTGAC CAAGATGCCC AGAGACTAGA CCCTATGCTT GTGAGTCACA 60
GTCATCAGAC ACGGCAAACC TCCAGTCTTC CTGACCTGTT GCTTAGGGAC ACATCCCGTT 120
GCTGCCCCTG ACGTCTGCCT GGTCAACCTT GACTTCCTTT GAGAGTAAGG AAGGGGGCGG 180
GGACACGTTG AAATCATGCC ACCCAAGGCC GAATCGGAAT GAGCAGATGA CGCCAAGTTG 240

ACGTCAAAGA CAGAGGCGAC AGAAACTCTG CAGCCAGCTC TTGCCCCCGA GGAGCTCAGG	300
TTCCTGCAGG AGTCATTTTA GCTTAGTCTT CTGAAGGACA CAGATACC ATG GAA GAG Met Glu Glu 1	357
AAC ACG TTT GGG GTT CAG CAG ATC CAA CCC AAT GTA ATT TCT GTT CGT Asn Thr Phe Gly Val Gln Gln Ile Gln Pro Asn Val Ile Ser Val Arg 5 10 15	405
CTC TTC AAA CGC AAA GTG GGA GGT CTG GGC TTC CTG GTG AAG GAA CGG Leu Phe Lys Arg Lys Val Gly Gly Leu Gly Phe Leu Val Lys Glu Arg 20 25 30 35	453
GTC AGC AAG CCT CCC GTG ATC ATC TCA GAC CTG ATT CGA GGA GGT GCT Val Ser Lys Pro Pro Val Ile Ile Ser Asp Leu Ile Arg Gly Gly Ala 40 45 50	501
GCG GAG CAG AGC GGC CTT ATC CAA GCT GGA GAC ATC ATT CTC GCA GTC Ala Glu Gln Ser Gly Leu Ile Gln Ala Gly Asp Ile Ile Leu Ala Val 55 60 65	549
AAC GAT CGG CCC TTG GTA GAC CTC AGC TAT GAC AGT GCC CTG GAG GTT Asn Asp Arg Pro Leu Val Asp Leu Ser Tyr Asp Ser Ala Leu Glu Val 70 75 80	597
CTC AGG GGC ATT GCC TCT GAG ACC CAC GTG GTC CTC ATT CTG AGG GGC Leu Arg Gly Ile Ala Ser Glu Thr His Val Val Leu Ile Leu Arg Gly 85 90 95	645
CCT GAG GGC TTC ACT ACA CAT CTG GAG ACC ACC TTC ACA GGG GAT GGA Pro Glu Gly Phe Thr Thr His Leu Glu Thr Thr Phe Thr Gly Asp Gly 100 105 110 115	693
ACC CCC AAG ACC ATC CGG GTG ACC CAG CCC CTC GGT CCT CCC ACC AAA Thr Pro Lys Thr Ile Arg Val Thr Gln Pro Leu Gly Pro Pro Thr Lys 120 125 130	741
GCC GTC GAT CTG TCT CAC CAG CCT TCA GCC AGC AAA GAC CAG TCA TTA Ala Val Asp Leu Ser His Gln Pro Ser Ala Ser Lys Asp Gln Ser Leu 135 140 145	789
GCA GTA GAC AGA GTC ACA GGT CTG GGT AAT GGC CCT CAG CAT GCC CAA Ala Val Asp Arg Val Thr Gly Leu Gly Asn Gly Pro Gln His Ala Gln 150 155 160	837
GGC CAT GGG CAG GGA GCT GGC TCA GTC TCC CAA GCT AAT GGT GTG GCC Gly His Gly Gln Gly Ala Gly Ser Val Ser Gln Ala Asn Gly Val Ala 165 170 175	885
ATT GAC CCC ACG ATG AAA AGC ACC AAG GCC AAC CTC CAG GAC ATC GGG Ile Asp Pro Thr Met Lys Ser Thr Lys Ala Asn Leu Gln Asp Ile Gly 180 185 190 195	933
GAA CAT GAT GAA CTG CTC AAA GAG ATA GAA CCT GTG CTG AGC ATC CTC Glu His Asp Glu Leu Leu Lys Glu Ile Glu Pro Val Leu Ser Ile Leu 200 205 210	981
AAC AGT GGG AGC AAA GCC ACC AAC AGA GGG GGA CCA GCC AAA GCA GAG Asn Ser Gly Ser Lys Ala Thr Asn Arg Gly Gly Pro Ala Lys Ala Glu 215 220 225	1029
ATG AAA GAC ACA GGA ATC CAG GTG GAC AGA GAC CTC GAT GGC AAA TCG Met Lys Asp Thr Gly Ile Gln Val Asp Arg Asp Leu Asp Gly Lys Ser 230 235 240	1077
CAC AAA GCT CCG CCC CTG GGC GGG GAC AAT GAC CGC GTC TTC AAT GAC	1125

His	Lys	Ala	Pro	Pro	Leu	Gly	Gly	Asp	Asn	Asp	Arg	Val	Phe	Asn	Asp	
	245					250					255					
CTG	TGG	GGG	AAG	GAC	AAC	GTT	CCT	GTG	ATC	CTT	AAC	AAC	CCG	TAT	TCA	1173
Leu	Trp	Gly	Lys	Asp	Asn	Val	Pro	Val	Ile	Leu	Asn	Asn	Pro	Tyr	Ser	
260					265					270					275	
GAG	AAG	GAA	CAG	TCC	CCT	ACC	TCG	GGG	AAA	CAG	TCT	CCC	ACC	AAG	AAC	1221
Glu	Lys	Glu	Gln	Ser	Pro	Thr	Ser	Gly	Lys	Gln	Ser	Pro	Thr	Lys	Asn	
				280					285					290		
GGC	AGC	CCT	TCC	AGG	TGC	CCC	CGT	TTC	CTC	AAG	GTC	AAG	AAC	TGG	GAG	1269
Gly	Ser	Pro	Ser	Arg	Cys	Pro	Arg	Phe	Leu	Lys	Val	Lys	Asn	Trp	Glu	
			295					300					305			
ACG	GAC	GTG	GTC	CTC	ACC	GAC	ACC	CTG	CAC	CTG	AAG	AGC	ACA	CTG	GAA	1317
Thr	Asp	Val	Val	Leu	Thr	Asp	Thr	Leu	His	Leu	Lys	Ser	Thr	Leu	Glu	
		310					315					320				
ACG	GGG	TGC	ACA	GAG	CAC	ATT	TGC	ATG	GGC	TCG	ATC	ATG	CTG	CCT	TCC	1365
Thr	Gly	Cys	Thr	Glu	His	Ile	Cys	Met	Gly	Ser	Ile	Met	Leu	Pro	Ser	
	325					330					335					
CAG	CAC	ACG	CGG	AAG	CCA	GAA	GAT	GTC	CGC	ACA	AAG	GAC	CAG	CTC	TTC	1413
Gln	His	Thr	Arg	Lys	Pro	Glu	Asp	Val	Arg	Thr	Lys	Asp	Gln	Leu	Phe	
340					345					350					355	
CCT	CTA	GCC	AAA	GAA	TTT	CTC	GAC	CAA	TAC	TAC	TCA	TCC	ATT	AAG	AGA	1461
Pro	Leu	Ala	Lys	Glu	Phe	Leu	Asp	Gln	Tyr	Tyr	Ser	Ser	Ile	Lys	Arg	
				360				365						370		
TTT	GGC	TCC	AAG	GCC	CAC	ATG	GAC	AGG	CTG	GAG	GAG	GTG	AAC	AAG	GAG	1509
Phe	Gly	Ser	Lys	Ala	His	Met	Asp	Arg	Leu	Glu	Glu	Val	Asn	Lys	Glu	
			375					380					385			
ATT	GAA	AGC	ACC	AGC	ACC	TAC	CAG	CTC	AAG	GAC	ACC	GAG	CTC	ATC	TAT	1557
Ile	Glu	Ser	Thr	Ser	Thr	Tyr	Gln	Leu	Lys	Asp	Thr	Glu	Leu	Ile	Tyr	
		390					395					400				
GGC	GCC	AAG	CAT	GCC	TGG	CGG	AAC	GCC	TCT	CGA	TGT	GTG	GGC	AGG	ATC	1605
Gly	Ala	Lys	His	Ala	Trp	Arg	Asn	Ala	Ser	Arg	Cys	Val	Gly	Arg	Ile	
405						410					415					
CAG	TGG	TCC	AAG	CTG	CAG	GTG	TTC	GAT	GCC	CGA	GAC	TGC	ACC	ACA	GCC	1653
Gln	Trp	Ser	Lys	Leu	Gln	Val	Phe	Asp	Ala	Arg	Asp	Cys	Thr	Thr	Ala	
420					425					430					435	
CAC	GGC	ATG	TTC	AAC	TAC	ATC	TGT	AAC	CAT	GTC	AAG	TAT	GCC	ACC	AAC	1701
His	Gly	Met	Phe	Asn	Tyr	Ile	Cys	Asn	His	Val	Lys	Tyr	Ala	Thr	Asn	
				440					445					450		
AAA	GGG	AAT	CTC	AGG	TCG	GCC	ATC	ACG	ATA	TTC	CCT	CAG	AGG	ACT	GAC	1749
Lys	Gly	Asn	Leu	Arg	Ser	Ala	Ile	Thr	Ile	Phe	Pro	Gln	Arg	Thr	Asp	
			455					460					465			
GGC	AAA	CAT	GAC	TTC	CGA	GTG	TGG	AAC	TCG	CAG	CTC	ATC	CGC	TAC	GCG	1797
Gly	Lys	His	Asp	Phe	Arg	Val	Trp	Asn	Ser	Gln	Leu	Ile	Arg	Tyr	Ala	
		470					475					480				
GGC	TAC	AAG	CAG	CCA	GAT	GGC	TCT	ACC	TTG	GGG	GAT	CCA	GCC	AAT	GTG	1845
Gly	Tyr	Lys	Gln	Pro	Asp	Gly	Ser	Thr	Leu	Gly	Asp	Pro	Ala	Asn	Val	
	485					490					495					
CAG	TTC	ACG	GAG	ATC	TGT	ATA	CAG	CAG	GGC	TGG	AAA	GCC	CCA	AGA	GGC	1893
Gln	Phe	Thr	Glu	Ile	Cys	Ile	Gln	Gln	Gly	Trp	Lys	Ala	Pro	Arg	Gly	
500					505					510					515	

CGC TTC GAC GTG CTG CCT CTC CTG CTT CAG GCC AAT GGC AAT GAC CCT Arg Phe Asp Val Leu Pro Leu Leu Leu Gln Ala Asn Gly Asn Asp Pro 520 525 530	1941
GAG CTC TTC CAG ATC CCC CCA GAG CTG GTG CTG GAA GTG CCC ATC AGG Glu Leu Phe Gln Ile Pro Pro Glu Leu Val Leu Glu Val Pro Ile Arg 535 540 545	1989
CAC CCC AAG TTC GAC TGG TTT AAG GAC CTG GGG CTC AAA TGG TAT GGC His Pro Lys Phe Asp Trp Phe Lys Asp Leu Gly Leu Lys Trp Tyr Gly 550 555 560	2037
CTC CCC GCT GTG TCC AAC ATG CTG CTG GAG ATC GGG GGC CTG GAG TTC Leu Pro Ala Val Ser Asn Met Leu Leu Glu Ile Gly Gly Leu Glu Phe 565 570 575	2085
AGC GCC TGT CCC TTC AGC GGC TGG TAC ATG GGC ACA GAG ATC GGC GTC Ser Ala Cys Pro Phe Ser Gly Trp Tyr Met Gly Thr Glu Ile Gly Val 580 585 590 595	2133
CGT GAC TAC TGT GAC AAC TCT CGA TAC AAC ATC CTG GAG GAA GTA GCC Arg Asp Tyr Cys Asp Asn Ser Arg Tyr Asn Ile Leu Glu Glu Val Ala 600 605 610	2181
AAG AAG ATG GAT TTG GAC ATG AGG AAG ACC TCG TCC CTC TGG AAG GAC Lys Lys Met Asp Leu Asp Met Arg Lys Thr Ser Ser Leu Trp Lys Asp 615 620 625	2229
CAA GCA CTG GTG GAG ATC AAC ATT GCT GTT CTA TAT AGC TTC CAG AGT Gln Ala Leu Val Glu Ile Asn Ile Ala Val Leu Tyr Ser Phe Gln Ser 630 635 640	2277
GAC AAG GTG ACC ATC GTT GAC CAC CAC TCT GCC ACG GAG TCC TTC ATC Asp Lys Val Thr Ile Val Asp His His Ser Ala Thr Glu Ser Phe Ile 645 650 655	2325
AAA CAC ATG GAG AAT GAA TAC CGC TGC AGA GGC GGC TGC CCC GCC GAC Lys His Met Glu Asn Glu Tyr Arg Cys Arg Gly Gly Cys Pro Ala Asp 660 665 670 675	2373
TGG GTG TGG ATT GTG CCT CCC ATG TCG GGC AGC ATC ACC CCT GTC TTC Trp Val Trp Ile Val Pro Pro Met Ser Gly Ser Ile Thr Pro Val Phe 680 685 690	2421
CAC CAG GAG ATG CTC AAC TAT AGA CTC ACC CCG TCC TTT GAA TAC CAG His Gln Glu Met Leu Asn Tyr Arg Leu Thr Pro Ser Phe Glu Tyr Gln 695 700 705	2469
CCT GAT CCA TGG AAC ACC CAC GTG TGG AAG GGC ACC AAC GGG ACC CCC Pro Asp Pro Trp Asn Thr His Val Trp Lys Gly Thr Asn Gly Thr Pro 710 715 720	2517
ACG AAG CGG CGA GCT ATC GGC TTT AAG AAA TTG GCA GAG GCC GTC AAG Thr Lys Arg Arg Ala Ile Gly Phe Lys Lys Leu Ala Glu Ala Val Lys 725 730 735	2565
TTC TCA GCC AAG CTA ATG GGC CAG GCC ATG GCC AAG AGG GTC AAG GCG Phe Ser Ala Lys Leu Met Gly Gln Ala Met Ala Lys Arg Val Lys Ala 740 745 750 755	2613
ACC ATT CTC TAC GCC ACA GAG ACA GGC AAA TCA CAA GCC TAT GCC AAG Thr Ile Leu Tyr Ala Thr Glu Thr Gly Lys Ser Gln Ala Tyr Ala Lys 760 765 770	2661
ACC CTG TGT GAG ATC TTC AAG CAC GCC TTC GAT GCC AAG GCA ATG TCC Thr Leu Cys Glu Ile Phe Lys His Ala Phe Asp Ala Lys Ala Met Ser 775 780 785	2709

ATG	GAG	GAG	TAT	GAC	ATC	GTG	CAC	CTG	GAG	CAC	GAA	GCC	CTG	GTC	TTG	2757
Met	Glu	Glu	Tyr	Asp	Ile	Val	His	Leu	Glu	His	Glu	Ala	Leu	Val	Leu	
		790					795					800				
GTG	GTC	ACC	AGC	ACC	TTT	GGC	AAT	GGA	GAC	CCC	CCT	GAG	AAC	GGG	GAG	2805
Val	Val	Thr	Ser	Thr	Phe	Gly	Asn	Gly	Asp	Pro	Pro	Glu	Asn	Gly	Glu	
		805				810					815					
AAA	TTC	GGC	TGT	GCT	TTA	ATG	GAG	ATG	AGG	CAC	CCC	AAC	TCT	GTG	CAG	2853
Lys	Phe	Gly	Cys	Ala	Leu	Met	Glu	Met	Arg	His	Pro	Asn	Ser	Val	Gln	
		820			825					830					835	
GAG	GAG	AGA	AAG	AGC	TAC	AAG	GTC	CGA	TTC	AAC	AGC	GTC	TCC	TCC	TAT	2901
Glu	Glu	Arg	Lys	Ser	Tyr	Lys	Val	Arg	Phe	Asn	Ser	Val	Ser	Ser	Tyr	
				840					845						850	
TCT	GAC	TCC	CGA	AAG	TCA	TCG	GGC	GAC	GGA	CCC	GAC	CTC	AGA	GAC	AAC	2949
Ser	Asp	Ser	Arg	Lys	Ser	Ser	Gly	Asp	Gly	Pro	Asp	Leu	Arg	Asp	Asn	
			855					860					865			
TTT	GAA	AGT	ACT	GGA	CCC	CTG	GCC	AAT	GTG	AGG	TTC	TCA	GTG	TTC	GGC	2997
Phe	Glu	Ser	Thr	Gly	Pro	Leu	Ala	Asn	Val	Arg	Phe	Ser	Val	Phe	Gly	
		870					875					880				
CTC	GGC	TCT	CGG	GCG	TAC	CCC	CAC	TTC	TGT	GCC	TTT	GGG	CAT	GCG	GTG	3045
Leu	Gly	Ser	Arg	Ala	Tyr	Pro	His	Phe	Cys	Ala	Phe	Gly	His	Ala	Val	
		885				890					895					
GAC	ACC	CTC	CTG	GAG	GAA	CTG	GGA	GGG	GAG	AGG	ATT	CTG	AAG	ATG	AGG	3093
Asp	Thr	Leu	Leu	Glu	Glu	Leu	Gly	Gly	Glu	Arg	Ile	Leu	Lys	Met	Arg	
		900				905				910					915	
GAG	GGG	GAT	GAG	CTT	TGC	GGA	CAG	GAA	GAA	GCT	TTC	AGG	ACC	TGG	GCC	3141
Glu	Gly	Asp	Glu	Leu	Cys	Gly	Gln	Glu	Glu	Ala	Phe	Arg	Thr	Trp	Ala	
				920					925						930	
AAG	AAA	GTC	TTC	AAG	GCA	GCC	TGT	GAT	GTG	TTC	TGC	GTG	GGG	GAT	GAC	3189
Lys	Lys	Val	Phe	Lys	Ala	Ala	Cys	Asp	Val	Phe	Cys	Val	Gly	Asp	Asp	
			935					940					945			
GTC	AAC	ATC	GAG	AAG	CCG	AAC	AAC	TCC	CTC	ATT	AGC	AAT	GAC	CGA	AGC	3237
Val	Asn	Ile	Glu	Lys	Pro	Asn	Asn	Ser	Leu	Ile	Ser	Asn	Asp	Arg	Ser	
		950					955					960				
TGG	AAG	AGG	AAC	AAG	TTC	CGC	CTC	ACG	TAT	GTG	GCG	GAA	GCT	CCA	GAT	3285
Trp	Lys	Arg	Asn	Lys	Phe	Arg	Leu	Thr	Tyr	Val	Ala	Glu	Ala	Pro	Asp	
		965				970					975					
CTG	ACC	CAA	GGT	CTT	TCC	AAT	GTT	CAC	AAA	AAA	CGA	GTC	TCG	GCT	GCT	3333
Leu	Thr	Gln	Gly	Leu	Ser	Asn	Val	His	Lys	Lys	Arg	Val	Ser	Ala	Ala	
				980		985				990					995	
CGA	CTC	CTC	AGC	CGC	CAA	AAC	CTG	CAA	AGC	CCT	AAG	TTC	AGC	CGA	TCG	3381
Arg	Leu	Leu	Ser	Arg	Gln	Asn	Leu	Gln	Ser	Pro	Lys	Phe	Ser	Arg	Ser	
				1000					1005						1010	
ACC	ATC	TTC	GTG	CGT	CTC	CAC	ACC	AAC	GGG	AAT	CAG	GAG	CTG	CAG	TAC	3429
Thr	Ile	Phe	Val	Arg	Leu	His	Thr	Asn	Gly	Asn	Gln	Glu	Leu	Gln	Tyr	
			1015					1020							1025	
CAG	CCA	GGG	GAC	CAC	CTG	GGT	GTC	TTC	CCC	GGC	AAC	CAC	GAG	GAC	CTC	3477
Gln	Pro	Gly	Asp	His	Leu	Gly	Val	Phe	Pro	Gly	Asn	His	Glu	Asp	Leu	
		1030					1035					1040				
GTG	AAT	GCA	CTC	ATT	GAA	CGG	CTG	GAG	GAT	GCA	CCG	CCT	GCC	AAC	CAC	3525
Val	Asn	Ala	Leu	Ile	Glu	Arg	Leu	Glu	Asp	Ala	Pro	Pro	Ala	Asn	His	
		1045					1050				1055					

GTG GTG AAG GTG GAG ATG CTG GAG GAG AGG AAC ACT GCT CTG GGT GTC Val Val Lys Val Glu Met Leu Glu Glu Arg Asn Thr Ala Leu Gly Val 1060 1065 1070 1075	3573
ATC AGT AAT TGG AAG GAT GAA TCT CGC CTC CCA CCC TGC ACC ATC TTC Ile Ser Asn Trp Lys Asp Glu Ser Arg Leu Pro Pro Cys Thr Ile Phe 1080 1085 1090	3621
CAG GCC TTC AAG TAC TAC CTG GAC ATC ACC ACG CCG CCC ACG CCC CTG Gln Ala Phe Lys Tyr Tyr Leu Asp Ile Thr Thr Pro Pro Thr Pro Leu 1095 1100 1105	3669
CAG CTG CAG CAG TTC GCC TCT CTG GCC ACT AAT GAG AAA GAG AAG CAG Gln Leu Gln Gln Phe Ala Ser Leu Ala Thr Asn Glu Lys Glu Lys Gln 1110 1115 1120	3717
CGG TTG CTG GTC CTC AGC AAG GGG CTC CAG GAA TAT GAG GAG TGG AAG Arg Leu Leu Val Leu Ser Lys Gly Leu Gln Glu Tyr Glu Glu Trp Lys 1125 1130 1135	3765
TGG GGC AAG AAC CCC ACA ATG GTG GAG GTG CTG GAG GAG TTC CCG TCC Trp Gly Lys Asn Pro Thr Met Val Glu Val Leu Glu Glu Phe Pro Ser 1140 1145 1150 1155	3813
ATC CAG ATG CCG GCT ACA CTT CTC CTC ACT CAG CTG TCG CTG CTG CAG Ile Gln Met Pro Ala Thr Leu Leu Leu Thr Gln Leu Ser Leu Leu Gln 1160 1165 1170	3861
CCT CGC TAC TAC TCC ATC AGC TCC TCT CCA GAC ATG TAC CCC GAC GAG Pro Arg Tyr Tyr Ser Ile Ser Ser Ser Pro Asp Met Tyr Pro Asp Glu 1175 1180 1185	3909
GTG CAC CTC ACT GTG GCC ATC GTC TCC TAC CAC ACC CGA GAC GGA GAA Val His Leu Thr Val Ala Ile Val Ser Tyr His Thr Arg Asp Gly Glu 1190 1195 1200	3957
GGA CCA GTC CAC CAC GGG GTG TGC TCC TCC TGG CTC AAC AGA ATA CAG Gly Pro Val His His Gly Val Cys Ser Ser Trp Leu Asn Arg Ile Gln 1205 1210 1215	4005
GCT GAC GAT GTA GTC CCC TGC TTC GTG AGA GGT GCC CCT AGC TTC CAC Ala Asp Asp Val Val Pro Cys Phe Val Arg Gly Ala Pro Ser Phe His 1220 1225 1230 1235	4053
CTG CCT CGA AAC CCC CAG GTG CCT TGC ATC CTG GTT GGC CCA GGC ACT Leu Pro Arg Asn Pro Gln Val Pro Cys Ile Leu Val Gly Pro Gly Thr 1240 1245 1250	4101
GGC ATC GCA CCC TTC CGA AGC TTC TGG CAA CAG CGA CAA TTT GAC ATC Gly Ile Ala Pro Phe Arg Ser Phe Trp Gln Gln Arg Gln Phe Asp Ile 1255 1260 1265	4149
CAA CAC AAA GGA ATG AAT CCG TGC CCC ATG GTT CTG GTC TTC GGG TGT Gln His Lys Gly Met Asn Pro Cys Pro Met Val Leu Val Phe Gly Cys 1270 1275 1280	4197
CGA CAA TCC AAG ATA GAT CAT ATC TAC AGA GAG GAG ACC CTG CAG GCT Arg Gln Ser Lys Ile Asp His Ile Tyr Arg Glu Glu Thr Leu Gln Ala 1285 1290 1295	4245
AAG AAC AAG GGC GTC TTC AGA GAG CTG TAC ACT GCC TAT TCC CGG GAA Lys Asn Lys Gly Val Phe Arg Glu Leu Tyr Thr Ala Tyr Ser Arg Glu 1300 1305 1310 1315	4293
CCG GAC AGG CCA AAG AAA TAT GTA CAG GAC GTG CTG CAG GAA CAG CTG Pro Asp Arg Pro Lys Lys Tyr Val Gln Asp Val Leu Gln Glu Gln Leu 1320 1325 1330	4341

57

GCT GAG TCT GTG TAC CGC GCC CTG AAG GAG CAA GGA GGC CAC ATT TAT Ala Glu Ser Val Tyr Arg Ala Leu Lys Glu Gln Gly Gly His Ile Tyr 1335 1340 1345	4389
GTC TGT GGG GAC GTT ACC ATG GCC GCC GAT GTC CTC AAA GCC ATC CAG Val Cys Gly Asp Val Thr Met Ala Ala Asp Val Leu Lys Ala Ile Gln 1350 1355 1360	4437
CGC ATA ATG ACC CAG CAG GGG AAA CTC TCA GAG GAG GAC GCT GGT GTA Arg Ile Met Thr Gln Gln Gly Lys Leu Ser Glu Glu Asp Ala Gly Val 1365 1370 1375	4485
TTC ATC AGC AGG CTG AGG GAT GAC AAC CGG TAC CAC GAG GAC ATC TTT Phe Ile Ser Arg Leu Arg Asp Asp Asn Arg Tyr His Glu Asp Ile Phe 1380 1385 1390 1395	4533
GGA GTC ACC CTC AGA ACG TAT GAA GTG ACC AAC CGC CTT AGA TCT GAG Gly Val Thr Leu Arg Thr Tyr Glu Val Thr Asn Arg Leu Arg Ser Glu 1400 1405 1410	4581
TCC ATC GCC TTC ATC GAA GAG AGC AAA AAA GAC GCA GAT GAG GTT TTC Ser Ile Ala Phe Ile Glu Glu Ser Lys Lys Asp Ala Asp Glu Val Phe 1415 1420 1425	4629
AGC TCC TAACTGGATC CTCCTGCCCC CGTGCGTGCG ATGTGGCGGC TGCCCCAAGT Ser Ser 143	4685
CCCCAAGTAA GGGCGGCCGC AGGTTGACTA AATTCCGACA CACACGGCTG AACCGAGTGG	4745
CCCTGCTCTG CCTCTTGTC TGTGCTGTG TCCTGGTCCT TCTTCCTGCT CTGGGCTCTC	4805
TCAACCCAC CCCTGGGTTT TCTCCTTGAC TCTTGGGCTA CGATGCATCA CCCTTGTAAC	4865
CTGCAGTGGC TCTCACAAA CCGCATCCTC CCCACCCCCA CCCGATTGCT GCCAAGGGCA	4925
GGTTGCGGTG CATGGCTGTT GCTCCTGTTG TTGGGGTCTG AAGGTGGCTG GCGCTGGGCC	4985
TCAGGTCACC CTGAACCACT CCCTTGGCCA CTTAAGCCCC CTTCACCCT CTTTTTATGA	5045
TGGTGTGTTT GT	5057

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1429 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Glu Glu Asn Thr Phe Gly Val Gln Gln Ile Gln Pro Asn Val Ile 1 5 10 15
Ser Val Arg Leu Phe Lys Arg Lys Val Gly Gly Leu Gly Phe Leu Val 20 25 30
Lys Glu Arg Val Ser Lys Pro Pro Val Ile Ile Ser Asp Leu Ile Arg 35 40 45
Gly Gly Ala Ala Glu Gln Ser Gly Leu Ile Gln Ala Gly Asp Ile Ile 50 55 60
Leu Ala Val Asn Asp Arg Pro Leu Val Asp Leu Ser Tyr Asp Ser Ala

58

65	70	75	80
Leu Glu Val	Leu Arg Gly Ile Ala Ser	Glu Thr His Val Val	Leu Ile
	85	90	95
Leu Arg Gly	Pro Glu Gly Phe Thr	Thr His Leu Glu Thr	Thr Phe Thr
	100	105	110
Gly Asp Gly	Thr Pro Lys Thr	Ile Arg Val Thr Gln	Pro Leu Gly Pro
	115	120	125
Pro Thr Lys	Ala Val Asp Leu Ser His	Gln Pro Ser Ala Ser	Lys Asp
	130	135	140
Gln Ser Leu	Ala Val Asp Arg Val Thr	Gly Leu Gly Asn Gly	Pro Gln
	145	150	155
His Ala Gln	Gly His Gly Gln Gly	Ala Gly Ser Val Ser	Gln Ala Asn
	165	170	175
Gly Val Ala	Ile Asp Pro Thr Met	Lys Ser Thr Lys Ala	Asn Leu Gln
	180	185	190
Asp Ile Gly	Glu His Asp Glu Leu Leu	Lys Glu Ile Glu	Pro Val Leu
	195	200	205
Ser Ile Leu	Asn Ser Gly Ser Lys Ala	Thr Asn Arg Gly Gly	Pro Ala
	210	215	220
Lys Ala Glu	Met Lys Asp Thr Gly Ile	Gln Val Asp Arg Asp	Leu Asp
	225	230	235
Gly Lys Ser	His Lys Ala Pro Pro	Leu Gly Gly Asp Asn	Asp Arg Val
	245	250	255
Phe Asn Asp	Leu Trp Gly Lys Asp	Asn Val Pro Val Ile	Leu Asn Asn
	260	265	270
Pro Tyr Ser	Glu Lys Glu Gln Ser	Pro Thr Ser Gly Lys	Gln Ser Pro
	275	280	285
Thr Lys Asn	Gly Ser Pro Ser Arg Cys	Pro Arg Phe Leu Lys	Val Lys
	290	295	300
Asn Trp Glu	Thr Asp Val Val Leu Thr	Asp Thr Leu His Leu	Lys Ser
	305	310	315
Thr Leu Glu	Thr Gly Cys Thr Glu His	Ile Cys Met Gly Ser	Ile Met
	325	330	335
Leu Pro Ser	Gln His Thr Arg Lys	Pro Glu Asp Val Arg	Thr Lys Asp
	340	345	350
Gln Leu Phe	Pro Leu Ala Lys Glu Phe	Leu Asp Gln Tyr Tyr	Ser Ser
	355	360	365
Ile Lys Arg	Phe Gly Ser Lys Ala His	Met Asp Arg Leu Glu	Glu Val
	370	375	380
Asn Lys Glu	Ile Glu Ser Thr Ser Thr	Tyr Gln Leu Lys Asp	Thr Glu
	385	390	395
Leu Ile Tyr	Gly Ala Lys His Ala Trp	Arg Asn Ala Ser Arg	Cys Val
	405	410	415
Gly Arg Ile	Gln Trp Ser Lys Leu Gln	Val Phe Asp Ala Arg	Asp Cys
	420	425	430

Thr Thr Ala His Gly Met Phe Asn Tyr Ile Cys Asn His Val Lys Tyr
 435 440 445
 Ala Thr Asn Lys Gly Asn Leu Arg Ser Ala Ile Thr Ile Phe Pro Gln
 450 455 460
 Arg Thr Asp Gly Lys His Asp Phe Arg Val Trp Asn Ser Gln Leu Ile
 465 470 475 480
 Arg Tyr Ala Gly Tyr Lys Gln Pro Asp Gly Ser Thr Leu Gly Asp Pro
 485 490 495
 Ala Asn Val Gln Phe Thr Glu Ile Cys Ile Gln Gln Gly Trp Lys Ala
 500 505 510
 Pro Arg Gly Arg Phe Asp Val Leu Pro Leu Leu Leu Gln Ala Asn Gly
 515 520 525
 Asn Asp Pro Glu Leu Phe Gln Ile Pro Pro Glu Leu Val Leu Glu Val
 530 535 540
 Pro Ile Arg His Pro Lys Phe Asp Trp Phe Lys Asp Leu Gly Leu Lys
 545 550 555 560
 Trp Tyr Gly Leu Pro Ala Val Ser Asn Met Leu Leu Glu Ile Gly Gly
 565 570 575
 Leu Glu Phe Ser Ala Cys Pro Phe Ser Gly Trp Tyr Met Gly Thr Glu
 580 585 590
 Ile Gly Val Arg Asp Tyr Cys Asp Asn Ser Arg Tyr Asn Ile Leu Glu
 595 600 605
 Glu Val Ala Lys Lys Met Asp Leu Asp Met Arg Lys Thr Ser Ser Leu
 610 615 620
 Trp Lys Asp Gln Ala Leu Val Glu Ile Asn Ile Ala Val Leu Tyr Ser
 625 630 635 640
 Phe Gln Ser Asp Lys Val Thr Ile Val Asp His His Ser Ala Thr Glu
 645 650 655
 Ser Phe Ile Lys His Met Glu Asn Glu Tyr Arg Cys Arg Gly Gly Cys
 660 665 670
 Pro Ala Asp Trp Val Trp Ile Val Pro Pro Met Ser Gly Ser Ile Thr
 675 680 685
 Pro Val Phe His Gln Glu Met Leu Asn Tyr Arg Leu Thr Pro Ser Phe
 690 695 700
 Glu Tyr Gln Pro Asp Pro Trp Asn Thr His Val Trp Lys Gly Thr Asn
 705 710 715 720
 Gly Thr Pro Thr Lys Arg Arg Ala Ile Gly Phe Lys Lys Leu Ala Glu
 725 730 735
 Ala Val Lys Phe Ser Ala Lys Leu Met Gly Gln Ala Met Ala Lys Arg
 740 745 750
 Val Lys Ala Thr Ile Leu Tyr Ala Thr Glu Thr Gly Lys Ser Gln Ala
 755 760 765
 Tyr Ala Lys Thr Leu Cys Glu Ile Phe Lys His Ala Phe Asp Ala Lys
 770 775 780
 Ala Met Ser Met Glu Glu Tyr Asp Ile Val His Leu Glu His Glu Ala

60

785		790		795		800
Leu Val Leu Val Val Thr Ser Thr Phe Gly Asn Gly Asp Pro Pro Glu						
		805		810		815
Asn Gly Glu Lys Phe Gly Cys Ala Leu Met Glu Met Arg His Pro Asn						
		820		825		830
Ser Val Gln Glu Glu Arg Lys Ser Tyr Lys Val Arg Phe Asn Ser Val						
		835		840		845
Ser Ser Tyr Ser Asp Ser Arg Lys Ser Ser Gly Asp Gly Pro Asp Leu						
		850		855		860
Arg Asp Asn Phe Glu Ser Thr Gly Pro Leu Ala Asn Val Arg Phe Ser						
		865		870		875
Val Phe Gly Leu Gly Ser Arg Ala Tyr Pro His Phe Cys Ala Phe Gly						
		885		890		895
His Ala Val Asp Thr Leu Leu Glu Glu Leu Gly Gly Glu Arg Ile Leu						
		900		905		910
Lys Met Arg Glu Gly Asp Glu Leu Cys Gly Gln Glu Glu Ala Phe Arg						
		915		920		925
Thr Trp Ala Lys Lys Val Phe Lys Ala Ala Cys Asp Val Phe Cys Val						
		930		935		940
Gly Asp Asp Val Asn Ile Glu Lys Pro Asn Asn Ser Leu Ile Ser Asn						
		945		950		955
Asp Arg Ser Trp Lys Arg Asn Lys Phe Arg Leu Thr Tyr Val Ala Glu						
		965		970		975
Ala Pro Asp Leu Thr Gln Gly Leu Ser Asn Val His Lys Lys Arg Val						
		980		985		990
Ser Ala Ala Arg Leu Leu Ser Arg Gln Asn Leu Gln Ser Pro Lys Phe						
		995		1000		1005
Ser Arg Ser Thr Ile Phe Val Arg Leu His Thr Asn Gly Asn Gln Glu						
		1010		1015		1020
Leu Gln Tyr Gln Pro Gly Asp His Leu Gly Val Phe Pro Gly Asn His						
		1025		1030		1035
Glu Asp Leu Val Asn Ala Leu Ile Glu Arg Leu Glu Asp Ala Pro Pro						
		1045		1050		1055
Ala Asn His Val Val Lys Val Glu Met Leu Glu Glu Arg Asn Thr Ala						
		1060		1065		1070
Leu Gly Val Ile Ser Asn Trp Lys Asp Glu Ser Arg Leu Pro Pro Cys						
		1075		1080		1085
Thr Ile Phe Gln Ala Phe Lys Tyr Tyr Leu Asp Ile Thr Thr Pro Pro						
		1090		1095		1100
Thr Pro Leu Gln Leu Gln Gln Phe Ala Ser Leu Ala Thr Asn Glu Lys						
		1105		1110		1115
Glu Lys Gln Arg Leu Leu Val Leu Ser Lys Gly Leu Gln Glu Tyr Glu						
		1125		1130		1135
Glu Trp Lys Trp Gly Lys Asn Pro Thr Met Val Glu Val Leu Glu Glu						
		1140		1145		1150

Phe Pro Ser Ile Gln Met Pro Ala Thr Leu Leu Leu Thr Gln Leu Ser
 1155 1160 1165
 Leu Leu Gln Pro Arg Tyr Tyr Ser Ile Ser Ser Ser Pro Asp Met Tyr
 1170 1175 1180
 Pro Asp Glu Val His Leu Thr Val Ala Ile Val Ser Tyr His Thr Arg
 1185 1190 1195 1200
 Asp Gly Glu Gly Pro Val His His Gly Val Cys Ser Ser Trp Leu Asn
 1205 1210 1215
 Arg Ile Gln Ala Asp Asp Val Val Pro Cys Phe Val Arg Gly Ala Pro
 1220 1225 1230
 Ser Phe His Leu Pro Arg Asn Pro Gln Val Pro Cys Ile Leu Val Gly
 1235 1240 1245
 Pro Gly Thr Gly Ile Ala Pro Phe Arg Ser Phe Trp Gln Gln Arg Gln
 1250 1255 1260
 Phe Asp Ile Gln His Lys Gly Met Asn Pro Cys Pro Met Val Leu Val
 1265 1270 1275 1280
 Phe Gly Cys Arg Gln Ser Lys Ile Asp His Ile Tyr Arg Glu Glu Thr
 1285 1290 1295
 Leu Gln Ala Lys Asn Lys Gly Val Phe Arg Glu Leu Tyr Thr Ala Tyr
 1300 1305 1310
 Ser Arg Glu Pro Asp Arg Pro Lys Lys Tyr Val Gln Asp Val Leu Gln
 1315 1320 1325
 Glu Gln Leu Ala Glu Ser Val Tyr Arg Ala Leu Lys Glu Gln Gly Gly
 1330 1335 1340
 His Ile Tyr Val Cys Gly Asp Val Thr Met Ala Ala Asp Val Leu Lys
 1345 1350 1355 1360
 Ala Ile Gln Arg Ile Met Thr Gln Gln Gly Lys Leu Ser Glu Glu Asp
 1365 1370 1375
 Ala Gly Val Phe Ile Ser Arg Leu Arg Asp Asp Asn Arg Tyr His Glu
 1380 1385 1390
 Asp Ile Phe Gly Val Thr Leu Arg Thr Tyr Glu Val Thr Asn Arg Leu
 1395 1400 1405
 Arg Ser Glu Ser Ile Ala Phe Ile Glu Glu Ser Lys Lys Asp Ala Asp
 1410 1415 1420
 Glu Val Phe Ser Ser
 1425

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5086 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: human bcl-2 cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1459..2178

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CGCCCCGCC	CTCCGCGCCG	CCTGCCCCGC	CGCCCCCGCG	GCTCCCCGCC	GCCGCTCTCC	60
GTGGCCCCGC	CGCGCTGCCG	CCGCGCCGCG	TGCCAGCGAA	GGTGCCGGGG	CTCCGGGCCC	120
TCCCTGCCGG	CGGCCGTCAG	CGCTCGGAGC	GAAGTGC	ACGGGAGGTC	CGGGAGGCGA	180
CCGTAGTCGC	GCCGCCGCGC	AGGACCAGGA	GGAGGAGAAA	GGGTGCGCAG	CCCGGAGGCG	240
GGGTGCGCCG	GTGGGGTGCA	GCGGAAGAGG	GGGTCCAGGG	GGGAGAACTT	CGTAGCAGTC	300
ATCCTTTTTTA	GGAAAAGAGG	GAAAAAATAA	AACCCTCCCC	CACCACCTCC	TTCTCCCCAC	360
CCCTCGCCGC	ACCACACACA	GCGCGGGCTT	CTAGCGCTCG	GCACCGGCGG	GCCAGGCGCG	420
TCCTGCCTTC	ATTTATCCAG	CAGCTTTTCG	GAAAATGCAT	TTGCTGTTCG	GAGTTTAATC	480
AGAAGACGAT	TCCTGCCTCC	GTCCCCGGCT	CCTTCATCGT	CCCATCTCCC	CTGTCTCTCT	540
CCTGGGGAGG	CGTGAAGCGG	TCCCGTGGAT	AGAGATTCAT	GCCTGTGTCC	GCGCGTGTGT	600
GCGCGCGTAT	AAATTGCCGA	GAAGGGGAAA	ACATCACAGG	ACTTCTGCGA	ATACCGGACT	660
GAAAATTGTA	ATTCATCTGC	CGCCGCCGCT	GCCAAAAAAA	AACTCGAGCT	CTTGAGATCT	720
CCGGTTGGGA	TTCTGCGGA	TTGACATTTT	TGTGAAGCAG	AAGTCTGGGA	ATCGATCTGG	780
AAATCCTCCT	AATTTTACT	CCCTCTCCCC	CCGACTCCTG	ATTCATTGGG	AAGTTTCAA	840
TCAGCTATAA	CTGGAGAGTG	CTGAAGATTG	ATGGGATCGT	TGCCTTATGC	ATTTGTTTTG	900
GTTTTACAAA	AAGGAACTT	GACAGAGGAT	CATGCTGTAC	TTAAAAATA	CAAGTAAGTC	960
TCGCACAGGA	AATTGGTTTA	ATGTAACCTT	CAATGGAAAC	CTTTGAGATT	TTTTACTTAA	1020
AGTGCATTCTG	AGTAAATTTA	ATTTCCAGGC	AGCTTAATAC	ATTGTTTTTA	GCCGTGTTAC	1080
TTGTAGTGTG	TATGCCCTGC	TTTCACTCAG	TGTGTACAGG	GAAACGCACC	TGATTTTTTA	1140
CTTATTAGTT	TGTTTTTCT	TTAACCTTTC	AGCATCACAG	AGGAAGTAGA	CTGATATTAA	1200
CAATACTTAC	TAATAATAAC	GTGCCTCATG	AAATAAAGAT	CCGAAAGGAA	TTGGAATAAA	1260
AATTTCTGTC	GTCTCATGCC	AAGAGGGAAA	CACCAGAATC	AAGTGTTCCG	CGTGATTGAA	1320
GACACCCCT	CGTCCAAGAA	TGCAAAGCAC	ATCCAATAAA	ATAGCTGGAT	TATAACTCCT	1380
CTTCTTTCTC	TGGGGGCCGT	GGGGTGGGAG	CTGGGGCGAG	AGGTGCCGTT	GGCCCCCGTT	1440
GCTTTTCCTC	TGGGAAGG	ATG GCG CAC GCT GGG AGA ACG GGG TAC GAC AAC				1491
		Met Ala His Ala Gly Arg Thr Gly Tyr Asp Asn				
		1 5 10				
CGG GAG ATA GTG ATG AAG TAC ATC CAT TAT AAG CTG TCG CAG AGG GGC						1539
Arg Glu Ile Val Met Lys Tyr Ile His Tyr Lys Leu Ser Gln Arg Gly						
		15 20 25				

TAC GAG TGG GAT GCG GGA GAT GTG GGC GCC GCG CCC CCG GGG GCC GCC	1587
Tyr Glu Trp Asp Ala Gly Asp Val Gly Ala Ala Pro Pro Gly Ala Ala	
30 35 40	
CCC GCA CCG GGC ATC TTC TCC TCC CAG CCC GGG CAC ACG CCC CAT CCA	1635
Pro Ala Pro Gly Ile Phe Ser Ser Gln Pro Gly His Thr Pro His Pro	
45 50 55	
GCC GCA TCC CGC GAC CCG GTC GCC AGG ACC TCG CCG CTG CAG ACC CCG	1683
Ala Ala Ser Arg Asp Pro Val Ala Arg Thr Ser Pro Leu Gln Thr Pro	
60 65 70 75	
GCT GCC CCC GGC GCC GCC GCG GGG CCT GCG CTC AGC CCG GTG CCA CCT	1731
Ala Ala Pro Gly Ala Ala Ala Gly Pro Ala Leu Ser Pro Val Pro Pro	
80 85 90	
GTG GTC CAC CTG GCC CTC CGC CAA GCC GGC GAC GAC TTC TCC CGC CGC	1779
Val Val His Leu Ala Leu Arg Gln Ala Gly Asp Asp Phe Ser Arg Arg	
95 100 105	
TAC CGC GGC GAC TTC GCC GAG ATG TCC AGC CAG CTG CAC CTG ACG CCC	1827
Tyr Arg Gly Asp Phe Ala Glu Met Ser Ser Gln Leu His Leu Thr Pro	
110 115 120	
TTC ACC GCG CGG GGA CGC TTT GCC ACG GTG GTG GAG GAG CTC TTC AGG	1875
Phe Thr Ala Arg Gly Arg Phe Ala Thr Val Val Glu Glu Leu Phe Arg	
125 130 135	
GAC GGG GTG AAC TGG GGG AGG ATT GTG GCC TTC TTT GAG TTC GGT GGG	1923
Asp Gly Val Asn Trp Gly Arg Ile Val Ala Phe Phe Glu Phe Gly Gly	
140 145 150 155	
GTC ATG TGT GTG GAG AGC GTC AAC CGG GAG ATG TCG CCC CTG GTG GAC	1971
Val Met Cys Val Glu Ser Val Asn Arg Glu Met Ser Pro Leu Val Asp	
160 165 170	
AAC ATC GCC CTG TGG ATG ACT GAG TAC CTG AAC CGG CAC CTG CAC ACC	2019
Asn Ile Ala Leu Trp Met Thr Glu Tyr Leu Asn Arg His Leu His Thr	
175 180 185	
TGG ATC CAG GAT AAC GGA GGC TGG GAT GCC TTT GTG GAA CTG TAC GGC	2067
Trp Ile Gln Asp Asn Gly Gly Trp Asp Ala Phe Val Glu Leu Tyr Gly	
190 195 200	
CCC AGC ATG CGG CCT CTG TTT GAT TTC TCC TGG CTG TCT CTG AAG ACT	2115
Pro Ser Met Arg Pro Leu Phe Asp Phe Ser Trp Leu Ser Leu Lys Thr	
205 210 215	
CTG CTC AGT TTG GCC CTG GTG GGA GCT TGC ATC ACC CTG GGT GCC TAT	2163
Leu Leu Ser Leu Ala Leu Val Gly Ala Cys Ile Thr Leu Gly Ala Tyr	
220 225 230 235	
CTG AGC CAC AAG TGAAGTCAAC ATGCCTGCCC CAAACAAATA TGCAAAAGGT	2215
Leu Ser His Lys	
240	
TCACTAAAGC AGTAGAATA ATATGCATTG TCAGTGATGT ACCATGAAAC AAAGCTGCAG	2275
GCTGTTTAAAG AAAAAATAAC ACACATATAA ACATCACACA CACAGACAGA CACACACACA	2335
CACAACAATT AACAGTCTTC AGGCAAAACG TCGAATCAGC TATTTACTGC CAAAGGGAAA	2395
TATCATTTAT TTTTACATT ATTAAGAAAA AAGATTTATT TATTTAAGAC AGTCCCATCA	2455
AAACTCCGTC TTTGGAAATC CGACCACTAA TTGCCAAACA CCGCTTCGTG TGGCTCCACC	2515
TGGATGTTCT GTGCCTGTAA ACATAGATTC GCTTTCCATG TTGTTGGCCG GATCACCATC	2575

TGAAGAGCAG	ACGGATGGAA	AAAGGACCTG	ATCATTGGGG	AAGCTGGCTT	TCTGGCTGCT	2635
GGAGGCTGGG	GAGAAGGTGT	TCATTCACTT	GCATTTCTTT	GCCCTGGGGG	CGTGATATTA	2695
ACAGAGGGAG	GGTTCCCGTG	GGGGGAAGTC	CATGCCTCCC	TGGCCTGAAG	AAGAGACTCT	2755
TTGCATATGA	CTCACATGAT	GCATACCTGG	TGGGAGGAAA	AGAGTTGGGA	ACTTCAGATG	2815
GACCTAGTAC	CCACTGAGAT	TTCCACGCCG	AAGGACAGCG	ATGGGAAAAA	TGCCCTTAAA	2875
TCATAGGAAA	GTATTTTTTT	AAGCTACCAA	TTGTGCCGAG	AAAAGCATT	TAGCAATTTA	2935
TACAATATCA	TCCAGTACCT	TAAACCCTGA	TTGTGTATAT	TCATATATTT	TGGATACGCA	2995
CCCCCCTACT	CCCAATACTG	GCTCTGTCTG	AGTAAGAAAC	AGAATCCTCT	GGAACCTGAG	3055
GAAGTGAACA	TTTCGGTGAC	TTCCGATCAG	GAAGGCTAGA	GTTACCCAGA	GCATCAGGCC	3115
GCCACAAGTG	CCTGCTTTTA	GGAGACCGAA	GTCCGCAGAA	CCTACCTGTG	TCCCAGCTTG	3175
GAGGCCTGGT	CCTGGAAGTG	AGCCGGGCCC	TACTGGCCT	CCTCCAGGGA	TGATCAACAG	3235
GGTAGTGTGG	TCTCCGAATG	TCTGGAAGCT	GATGGATGGA	GCTCAGAATT	CCACTGTCAA	3295
GAAAGAGCAG	TAGAGGGGTG	TGGCTGGGCC	TGTCACCCTG	GGGCCCTCCA	GGTAGGCCCC	3355
TTTTCACGTG	GAGCATAGGA	GCCACGACCC	TTCTTAAGAC	ATGTATCACT	GTAGAGGGAA	3415
GGAACAGAGG	CCCTGGGCCT	TCCTATCAGA	AGGACATGGT	GAAGGCTGGG	AACGTGAGGA	3475
GAGGCAATGG	CCACGGCCCA	TTTTGGCTGT	AGCACATGGC	ACGTTGGCTG	TGTGGCCTTG	3535
GCCACCTGTG	AGTTTAAAGC	AAGGCTTTAA	ATGACTTTGG	AGAGGGTCAC	AAATCCTAAA	3595
AGAAGCATTG	AAGTGAGGTG	TCATGGATTA	ATTGACCCCT	GTCTATGGAA	TTACATGTAA	3655
AACATTATCT	TGTCACGTG	GTTTGGTTTT	ATTTGAAAAC	CTGACAAAAA	AAAAGTTCCA	3715
GGTGTGGAAT	ATGGGGGTTA	TCTGTACATC	CTGGGGCATT	AAAAAAAAAT	CAATGGTGGG	3775
GAACATATAA	GAAGTAACAA	AAGAAGTGAC	ATCTTCAGCA	AATAAACTAG	GAAATTTTTT	3835
TTTCTTCCAG	TTTAGAATCA	GCCTTGAAAC	ATTGATGGAA	TAACTCTGTG	GCATTATTGC	3895
ATTATATACC	ATTTATCTGT	ATTAACCTTG	GAATGTACTC	TGTTCAATGT	TTAATGCTGT	3955
GGTTGATATT	TCGAAAGCTG	CTTTAAAAA	ATACATGCAT	CTCAGCGTTT	TTTTGTTTTT	4015
AATTGTATTT	AGTTATGGCC	TATACACTAT	TTGTGAGCAA	AGGTGATCGT	TTTCTGTTTG	4075
AGATTTTTAT	CTCTTGATTC	TTCAAAAGCA	TTCTGAGAAG	GTGAGATAAG	CCCTGAGTCT	4135
CAGCTACCTA	AGAAAAACCT	GGATGTCACT	GGCCACTGAG	GAGCTTTGTT	TCAACCAAGT	4195
CATGTGCATT	TCCACGTCAA	CAGAATTGTT	TATTGTGACA	GTTATATCTG	TTGTCCCTTT	4255
GACCTTGTTT	CTTGAAGGTT	TCCTCGTCCC	TGGGCAATTC	CGCATTTAAT	TCATGGTATT	4315
CAGGATTACA	TGCATGTTTG	GTTAAACCCA	TGAGATTCAT	TCAGTTAAAA	ATCCAGATGG	4375
CGAATGACCA	GCAGATTCAA	ATCTATGGTG	GTTTGACCTT	TAGAGAGTTG	CTTTACGTGG	4435
CCTGTTTCAA	CACAGACCCA	CCCAGAGCCC	TCCTGCCCTC	CTTCGCGGGG	GGCTTTCTCA	4495
TGGCTGTCCT	TCAGGGTCTT	CCTGAAATGC	AGTGTCGTT	ACGCTCCACC	AAGAAAGCAG	4555
GAAACCTGTG	GTATGAAGCC	AGACCTCCCC	GGCGGGCCTC	AGGGAACAGA	ATGATCAGAC	4615

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CTTTGAATGA TTCTAATTTT TAAGCAAAAT ATTATTTTAT GAAAGGTTTA CATTGTCAAA 4675
GTGATGAATA TGGAAATATCC AATCCTGTGC TGCTATCCTG CCAAATCAT TTTAATGGAG 4735
TCAGTTTGCA GTATGCTCCA CGTGGTAAGA TCCTCCAAGC TGCTTTAGAA GTAACAATGA 4795
AGAACGTGGA CGTTTTTAAT ATAAAGCCTG TTTTGTCTTT TGTGTGTGTT CAAACGGGAT 4855
TCACAGAGTA TTTGAAAAAT GTATATATAT TAAGAGGTCA CGGGGGCTAA TTGCTAGCTG 4915
GCTGCCCTTTT GCTGTGGGGT TTTGTTACCT GGTTTTAATA ACAGTAAATG TGCCAGCCT 4975
CTTGGCCCCA GAACTGTACA GTATTGTGGC TGCACTTGCT CTAAGAGTAG TTGATGTTGC 5035
ATTTTCCTTA TTGTTAAAAA CATGTTAGAA GCAATGAATG TATATAAAG C 5086

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(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 239 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

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Met Ala His Ala Gly Arg Thr Gly Tyr Asp Asn Arg Glu Ile Val Met
 1             5             10             15
Lys Tyr Ile His Tyr Lys Leu Ser Gln Arg Gly Tyr Glu Trp Asp Ala
          20             25             30
Gly Asp Val Gly Ala Ala Pro Pro Gly Ala Ala Pro Ala Pro Gly Ile
          35             40             45
Phe Ser Ser Gln Pro Gly His Thr Pro His Pro Ala Ala Ser Arg Asp
          50             55             60
Pro Val Ala Arg Thr Ser Pro Leu Gln Thr Pro Ala Ala Pro Gly Ala
          65             70             75             80
Ala Ala Gly Pro Ala Leu Ser Pro Val Pro Pro Val Val His Leu Ala
          85             90             95
Leu Arg Gln Ala Gly Asp Asp Phe Ser Arg Arg Tyr Arg Gly Asp Phe
          100             105             110
Ala Glu Met Ser Ser Gln Leu His Leu Thr Pro Phe Thr Ala Arg Gly
          115             120             125
Arg Phe Ala Thr Val Val Glu Glu Leu Phe Arg Asp Gly Val Asn Trp
          130             135             140
Gly Arg Ile Val Ala Phe Phe Glu Phe Gly Gly Val Met Cys Val Glu
          145             150             155             160
Ser Val Asn Arg Glu Met Ser Pro Leu Val Asp Asn Ile Ala Leu Trp
          165             170             175
Met Thr Glu Tyr Leu Asn Arg His Leu His Thr Trp Ile Gln Asp Asn
          180             185             190
Gly Gly Trp Asp Ala Phe Val Glu Leu Tyr Gly Pro Ser Met Arg Pro
          195             200             205

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Leu Phe Asp Phe Ser Trp Leu Ser Leu Lys Thr Leu Leu Ser Leu Ala
 210 215 220

Leu Val Gly Ala Cys Ile Thr Leu Gly Ala Tyr Leu Ser His Lys
 225 230 235

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1846 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: bcl-2 fusion gene; Seto, et al.,
EMBO J 7:123 (1988)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 887..1606

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

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ACCACCTCCT TCTCCCCACC CCTCGCCGCA CCACACACAG CGCGGGCTTC TGGCGCTCGG      60
CACCGGCGGG CCAGGCGCGT CCTGTCTTCA TTTATCCAGC AGCTTTTCGG AAAATCCATT      120
TGGTGTTTCGG AGTTTAATCA GAAGAGGATT CCTGCCTCCG TCCCCGGCTC CTTCATCGTC      180
CCCTCTCCCC TGTCTCTCTC CTGGGGAGGC GTGAAGAGAG ATTCATGCCT GTGCCCCGCGC      240
GTGTGTGCGC GCGTATAAAT TGCCGAGAAG GGGAAAACAT CACAGGACTT CTGCGAATAC      300
CGGACTGAAA ATTGTAGCTC ATCTGCCGCC GCCGCTGCCT TTTTTTTTTT TCGAGCTCTT      360
GAGATCTCCG GTTGGGACTC CTGCGGATTG ACATTTCTGT GAAGCAGAAG TCTGGGAATC      420
GATCTGGAAA TCCTCCTAAT TTTTACTCCC TCTCCCCCGG ACTCCTGATT CATTGGGAAG      480
TTTCAAATCA GCTATAACTG GAGAGAGCTG AAGATTGATG GGATCGTTGC CTTATGCCTT      540
TGTTTTGGTT TTACAAAAAG GAAACTTGAC AGAGGATCAT GCTATACTTA AAAAATACAA      600
CATCGCAGAG GAAGTAGACT CATATTAAAA ATACTTACTA ATAATAACGT GCCTCATGAA      660
GTAAAGATCC GAAAGGAATT GGAATAAAAC TTTCCTGCAT CTCAAGCCAA GGGGGAAACA      720
CCAGAATCAA GTGTTCCGCG TGATTGAAGA CACCCCTCG TCCAAGAATG CAAAGCACAT      780
CCAATAAAAG AGCTGGATTA TAACTCCTCT TCTTTCTCTG GGGGCCGTGG GGTAGGGGCT      840
GGGGCGAGAG GTGCCGTTGG CCCCCGTTGC TTTTCCTCTG GGAGGG ATG GCG CAC      895
                                     Met Ala His
                                     1
GCT GGG AGA AGT GGT TAC GAT AAC CGG GAG ATA GTG ATG AAG TAC ATC      943
Ala Gly Arg Ser Gly Tyr Asp Asn Arg Glu Ile Val Met Lys Tyr Ile
    5              10              15

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67

CAT TAT AAG CTG TCG CAG AGG GGC TAC GAG TGG GAT GCG GGA GAT GTG His Tyr Lys Leu Ser Gln Arg Gly Tyr Glu Trp Asp Ala Gly Asp Val 20 25 30 35	991
GGC GCC GCG CCC CCG GGG GCC GCC CCC GCA CCG GGC TTC TTC TCC TCC Gly Ala Ala Pro Pro Gly Ala Ala Pro Ala Pro Gly Phe Phe Ser Ser 40 45 50	1039
CAG CCC GGG CAC ACG CCC CAT CCA GCC GCA TCC CGG GAC CCG GTC GCC Gln Pro Gly His Thr Pro His Pro Ala Ala Ser Arg Asp Pro Val Ala 55 60 65	1087
AGG ACC TCG CCA CTA CAG ACC CCG GCT GCC CCC GGC GCC GCC GCG GGG Arg Thr Ser Pro Leu Gln Thr Pro Ala Ala Pro Gly Ala Ala Gly 70 75 80	1135
CCT GCG CTC AGC CCG GTG CCA CCT GTG GTC CAC CTG ACC CTC CGC CAG Pro Ala Leu Ser Pro Val Pro Pro Val Val His Leu Thr Leu Arg Gln 85 90 95	1183
GCC GGC GAC GAC TTC TCC CGC CGC TAC CGC CGC GAC TTC GCC GAG ATG Ala Gly Asp Asp Phe Ser Arg Arg Tyr Arg Arg Asp Phe Ala Glu Met 100 105 110 115	1231
TCC AGC CAG CTG CAC CTG ACG CCC TTC ACC GCG CGG GGA TGC TTT GCC Ser Ser Gln Leu His Leu Thr Pro Phe Thr Ala Arg Gly Cys Phe Ala 120 125 130	1279
ACG GTG GTG GAG GAG CTC TTC AGG GAC GGG GTG AAC TGG GGG AGG ATT Thr Val Val Glu Glu Leu Phe Arg Asp Gly Val Asn Trp Gly Arg Ile 135 140 145	1327
GTG GCC TTC TTT GAG TTC GGT GGG GTC ATG TGT GTG GAG AGC GTC AAC Val Ala Phe Phe Glu Phe Gly Gly Val Met Cys Val Glu Ser Val Asn 150 155 160	1375
CGG GAG ATG TCG CCC CTG GTG GAC AAC ATC GCC CTG TGG ATG ACT GAG Arg Glu Met Ser Pro Leu Val Asp Asn Ile Ala Leu Trp Met Thr Glu 165 170 175	1423
TAC CTG AAC CGG CAC CTG CAC ACC TGG ATC CAG GAT AAC GGA GGC TGG Tyr Leu Asn Arg His Leu His Thr Trp Ile Gln Asp Asn Gly Gly Trp 180 185 190 195	1471
GAT GCC TTT GTG GAA CTG TAC GGC CCC AGC ATG CGG CCT CTG TTT GAT Asp Ala Phe Val Glu Leu Tyr Gly Pro Ser Met Arg Pro Leu Phe Asp 200 205 210	1519
TTC TCC TGG CTG TCT CTG AAG ACT CTG CTC AGT TTG GCC CTG GTG GGA Phe Ser Trp Leu Ser Leu Lys Thr Leu Leu Ser Leu Ala Leu Val Gly 215 220 225	1567
GCT TGC ATC ACC CTG GGT GCC TAT CTG GGC CAC AAG TGAAGTCAAC Ala Cys Ile Thr Leu Gly Ala Tyr Leu Gly His Lys 230 235 240	1613
ATGCCTGCCC CAAACAAATA TGCAAAAGGT TCACTAAAGC AGTAGAAATA ATATGCATTG	1673
TCAGTGATGT ACCATGAAAC AAAGCTGCAG GCTGTTTAAG AAAAAATAAC ACACATATAA	1733
ACATCACACA CACAGACAGA CACACACACA CACAACAATT AACAGTCTTC AGGCAAAACG	1793
TCGAATCAGC TATTTACTGC CAAAGGGAAA TATCATTTAT TTTTACATT ATT	1846

(2) INFORMATION FOR SEQ ID NO:17:

68

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 239 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

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Met Ala His Ala Gly Arg Ser Gly Tyr Asp Asn Arg Glu Ile Val Met
 1           5           10           15
Lys Tyr Ile His Tyr Lys Leu Ser Gln Arg Gly Tyr Glu Trp Asp Ala
          20           25           30
Gly Asp Val Gly Ala Ala Pro Pro Gly Ala Ala Pro Ala Pro Gly Phe
          35           40           45
Phe Ser Ser Gln Pro Gly His Thr Pro His Pro Ala Ala Ser Arg Asp
          50           55           60
Pro Val Ala Arg Thr Ser Pro Leu Gln Thr Pro Ala Ala Pro Gly Ala
          65           70           75           80
Ala Ala Gly Pro Ala Leu Ser Pro Val Pro Pro Val Val His Leu Thr
          85           90           95
Leu Arg Gln Ala Gly Asp Asp Phe Ser Arg Arg Tyr Arg Arg Asp Phe
          100          105          110
Ala Glu Met Ser Ser Gln Leu His Leu Thr Pro Phe Thr Ala Arg Gly
          115          120          125
Cys Phe Ala Thr Val Val Glu Glu Leu Phe Arg Asp Gly Val Asn Trp
          130          135          140
Gly Arg Ile Val Ala Phe Phe Glu Phe Gly Gly Val Met Cys Val Glu
          145          150          155          160
Ser Val Asn Arg Glu Met Ser Pro Leu Val Asp Asn Ile Ala Leu Trp
          165          170          175
Met Thr Glu Tyr Leu Asn Arg His Leu His Thr Trp Ile Gln Asp Asn
          180          185          190
Gly Gly Trp Asp Ala Phe Val Glu Leu Tyr Gly Pro Ser Met Arg Pro
          195          200          205
Leu Phe Asp Phe Ser Trp Leu Ser Leu Lys Thr Leu Leu Ser Leu Ala
          210          215          220
Leu Val Gly Ala Cys Ile Thr Leu Gly Ala Tyr Leu Gly His Lys
          225          230          235

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(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4353 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: Human NOS-1 gene, Fujisawa, et al,
J. Neurochem 63:140 1994

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..4305

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATG GAG GAT CAC ATG TTC GGT GTT CAG CAA ATC CAG CCC AAT GTC ATT	48
Met Glu Asp His Met Phe Gly Val Gln Gln Ile Gln Pro Asn Val Ile	
1 5 10 15	
TCT GTT CGT CTC TTC AAG CGC AAA GTT GGG GGC CTG GGA TTT CTG GTG	96
Ser Val Arg Leu Phe Lys Arg Lys Val Gly Gly Leu Gly Phe Leu Val	
20 25 30	
AAG GAG CGG GTC AGT AAG CCG CCC GTG ATC ATC TCT GAC CTG ATT CGT	144
Lys Glu Arg Val Ser Lys Pro Pro Val Ile Ile Ser Asp Leu Ile Arg	
35 40 45	
GGG GGC GCC GCA GAG CAG AGT GGC CTC ATC CAG GCC GGA GAC ATC ATT	192
Gly Gly Ala Ala Glu Gln Ser Gly Leu Ile Gln Ala Gly Asp Ile Ile	
50 55 60	
CTT GCG GTC AAC GGC CGG CCC TTG GTG GAC CTG AGC TAT GAC AGC GCC	240
Leu Ala Val Asn Gly Arg Pro Leu Val Asp Leu Ser Tyr Asp Ser Ala	
65 70 75 80	
CTG GAG GTA CTC AGA GGC ATT GCC TCT GAG ACC CAC GTG GTC CTC ATT	288
Leu Glu Val Leu Arg Gly Ile Ala Ser Glu Thr His Val Val Leu Ile	
85 90 95	
CTG AGG GGC CCT GAA GGT TTC ACC ACG CAC CTG GAG ACC ACC TTT ACA	336
Leu Arg Gly Pro Glu Gly Phe Thr Thr His Leu Glu Thr Thr Phe Thr	
100 105 110	
GGT GAT GGG ACC CCC AAG ACC ATC CGG GTG ACA CAG CCC CTG GGT CCC	384
Gly Asp Gly Thr Pro Lys Thr Ile Arg Val Thr Gln Pro Leu Gly Pro	
115 120 125	
CCC ACC AAA GCC GTG GAT CTG TCC CAC CAG CCA CCG GCC GGC AAA GAA	432
Pro Thr Lys Ala Val Asp Leu Ser His Gln Pro Pro Ala Gly Lys Glu	
130 135 140	
CAG CCC CTG GCA GTG GAT GGG GCC TCG GGT CCC GGG AAT GGG CCT CAG	480
Gln Pro Leu Ala Val Asp Gly Ala Ser Gly Pro Gly Asn Gly Pro Gln	
145 150 155 160	
CAT GCC TAC GAT GAT GGG CAG GAG GCT GGC TCA CTC CCC CAT GCC AAC	528
His Ala Tyr Asp Asp Gly Gln Glu Ala Gly Ser Leu Pro His Ala Asn	
165 170 175	
GGC CTG GCC CCC AGG CCC CCA GGC CAG GAC CCC GCG AAG AAA GCA ACC	576
Gly Leu Ala Pro Arg Pro Pro Gly Gln Asp Pro Ala Lys Lys Ala Thr	
180 185 190	
AGA GTC AGC CTC CAA GGC AGA GGG GAG AAC AAT GAA CTG CTC AAG GAG	624
Arg Val Ser Leu Gln Gly Arg Gly Glu Asn Asn Glu Leu Leu Lys Glu	
195 200 205	
ATA GAG CCT GTG CTG AGC CTT CTC ACC AGT GGG AGC AGA GGG GTC AAG	672
Ile Glu Pro Val Leu Ser Leu Leu Thr Ser Gly Ser Arg Gly Val Lys	

210	215	220	
GGA GGG GCA CCT GCC AAG GCA GAG ATG AAA GAT ATG GGA ATC CAG GTG Gly Gly Ala Pro Ala Lys Ala Glu Met Lys Asp Met Gly Ile Gln Val 225 230 235 240			720
GAC AGA GAT TTG GAC GGC AAG TCA CAC AAA CCT CTG CCC CTC GGC GTG Asp Arg Asp Leu Asp Gly Lys Ser His Lys Pro Leu Pro Leu Gly Val 245 250 255			768
GAG AAC GAC CGA GTC TTC AAT GAC CTA TGG GGG AAG GGC AAT GTG CCT Glu Asn Asp Arg Val Phe Asn Asp Leu Trp Gly Lys Gly Asn Val Pro 260 265 270			816
GTC GTC CTC AAC AAC CCA TAT TCA GAG AAG GAG CAG CCC CCC ACC TCA Val Val Leu Asn Asn Pro Tyr Ser Glu Lys Glu Gln Pro Thr Ser 275 280 285			864
GGA AAA CAG TCC CCC ACA AAG AAT GGC AGC CCC TCC AAG TGT CCA CGC Gly Lys Gln Ser Pro Thr Lys Asn Gly Ser Pro Lys Cys Pro Arg 290 295 300			912
TTC CTC AAG GTC AAG AAC TGG GAG ACT GAG GTG GTT CTC ACT GAC ACC Phe Leu Lys Val Lys Asn Trp Glu Thr Glu Val Val Leu Thr Asp Thr 305 310 315 320			960
CTC CAC CTT AAG AGC ACA TTG GAA ACG GGA TGC ACT GAG TAC ATC TGC Leu His Leu Lys Ser Thr Leu Glu Thr Gly Cys Thr Glu Tyr Ile Cys 325 330 335			1008
ATG GGC TCC ATC ATG CAT CCT TCT CAG CAT GCA AGG AGG CCT GAA GAC Met Gly Ser Ile Met His Pro Ser Gln His Ala Arg Arg Pro Glu Asp 340 345 350			1056
GTC CGC ACA AAA GGA CAG CTC TTC CCT CTC GCC AAA GAG TTT ATT GAT Val Arg Thr Lys Gly Gln Leu Phe Pro Leu Ala Lys Glu Phe Ile Asp 355 360 365			1104
CAA TAC TAT TCA TCA ATT AAA AGA TTT GGC TCC AAA GCC CAC ATG GAA Gln Tyr Tyr Ser Ser Ile Lys Arg Phe Gly Ser Lys Ala His Met Glu 370 375 380			1152
AGG CTG GAA GAG GTG AAC AAA GAG ATC GAC ACC ACT AGC ACT TAC CAG Arg Leu Glu Glu Val Asn Lys Glu Ile Asp Thr Thr Ser Thr Tyr Gln 385 390 395 400			1200
CTC AAG GAC ACA GAG CTC ATC TAT GGG GCC AAG CAC GCC TGG CGG AAT Leu Lys Asp Thr Glu Leu Ile Tyr Gly Ala Lys His Ala Trp Arg Asn 405 410 415			1248
GCC TCG CGC TGT GTG GGC AGG ATC CAG TGG TCC AAG CTG CAG GTA TTC Ala Ser Arg Cys Val Gly Arg Ile Gln Trp Ser Lys Leu Gln Val Phe 420 425 430			1296
GAT GCC CGT GAC TGC ACC ACG GCC CAC GGG ATG TTC AAC TAC ATC TGT Asp Ala Arg Asp Cys Thr Thr Ala His Gly Met Phe Asn Tyr Ile Cys 435 440 445			1344
AAC CAT GTC AAG TAT GCC ACC AAC AAA GGG AAC CTC AGG TCT GCC ATC Asn His Val Lys Tyr Ala Thr Asn Lys Gly Asn Leu Arg Ser Ala Ile 450 455 460			1392
ACC ATA TTC CCC CAG AGG ACA GAC GGC AAG CAC GAC TTC CGA GTC TGG Thr Ile Phe Pro Gln Arg Thr Asp Gly Lys His Asp Phe Arg Val Trp 465 470 475 480			1440
AAC TCC CAG CTC ATC CGC TAC GCT GGC TAC AAG CAG CCT GAC GGC TCC			1488

Asn	Ser	Gln	Leu	Ile 485	Arg	Tyr	Ala	Gly	Tyr 490	Lys	Gln	Pro	Asp	Gly 495	Ser		
ACC	CTG	GGG	GAC	CCA	GCC	AAT	GTG	CAG	TTC	ACA	GAG	ATA	TGC	ATA	CAG	1536	
Thr	Leu	Gly	Asp 500	Pro	Ala	Asn	Val	Gln 505	Phe	Thr	Glu	Ile	Cys 510	Ile	Gln		
CAG	GGC	TGG	AAA	CCG	CCT	AGA	GGC	CGC	TTC	GAT	GTC	CTG	CCG	CTC	CTG	1584	
Gln	Gly	Trp	Lys 515	Pro	Pro	Arg	Gly 520	Arg	Phe	Asp	Val	Leu 525	Pro	Leu	Leu		
CTT	CAG	GCC	AAC	GGC	AAT	GAC	CCT	GAG	CTC	TTC	CAG	ATT	CCT	CCA	GAG	1632	
Leu	Gln	Ala	Asn 530	Gly	Asn	Asp 535	Pro	Glu	Leu	Phe	Gln 540	Ile	Pro	Pro	Glu		
CTG	GTG	TTG	GAA	GTT	CCC	ATC	AGG	CAC	CCC	AAG	TTT	GAG	TGG	TTC	AAG	1680	
Leu	Val	Leu	Glu 545	Val	Pro	Ile 550	Arg	His	Pro	Lys 555	Phe	Glu	Trp	Phe	Lys 560		
GAC	CTG	GGG	CTG	AAG	TGG	TAC	GGC	CTC	CCC	GCC	GTG	TCC	AAC	ATG	CTC	1728	
Asp	Leu	Gly	Leu 565	Lys	Trp	Tyr	Gly	Leu	Pro	Ala	Val	Ser	Asn	Met	Leu 575		
CTA	GAG	ATT	GGC	GGC	CTG	GAG	TTC	AGC	GCC	TGT	CCC	TTC	AGT	GGC	TGG	1776	
Leu	Glu	Ile	Gly 580	Gly	Leu	Glu	Phe	Ser 585	Ala	Cys	Pro	Phe	Ser 590	Gly	Trp		
TAC	ATG	GGC	ACA	GAG	ATT	GGT	GTC	CGC	GAC	TAC	TGT	GAC	AAC	TCC	CGC	1824	
Tyr	Met	Gly	Thr 595	Glu	Ile	Gly 600	Val	Arg	Asp	Tyr	Cys 605	Asn	Ser	Arg			
TAC	AAT	ATC	CTG	GAG	GAA	GTG	GCC	AAG	AAG	ATG	AAC	TTA	GAC	ATG	AGG	1872	
Tyr	Asn	Ile	Leu 610	Glu	Glu	Val 615	Ala	Lys	Lys	Met	Asn 620	Leu	Asp	Met	Arg		
AAG	ACG	TCC	TCC	CTG	TGG	AAG	GAC	CAG	GCG	CTG	GTG	GAG	ATC	AAT	ATC	1920	
Lys	Thr	Ser	Ser 625	Leu	Trp 630	Lys	Asp	Gln	Ala 635	Leu	Val	Glu	Ile	Asn 640	Ile		
GCG	GTT	CTC	TAT	AGC	TTC	CAG	AGT	GAC	AAA	GTG	ACC	ATT	GTT	GAC	CAT	1968	
Ala	Val	Leu	Tyr 645	Ser	Phe	Gln	Ser	Asp 650	Lys	Val	Thr	Ile	Val	Asp 655	His		
CAC	TCC	GCC	ACC	GAG	TCC	TTC	ATT	AAG	CAC	ATG	GAG	AAT	GAG	TAC	CGC	2016	
His	Ser	Ala	Thr 660	Glu	Ser	Phe	Ile	Lys 665	His	Met	Glu	Asn 670	Glu	Tyr	Arg		
TGC	CGG	GGG	GGC	TGC	CCT	GCC	GAC	TGG	GTG	TGG	ATC	GTG	CCC	CCC	ATG	2064	
Cys	Arg	Gly	Gly 675	Cys	Pro	Ala	Asp 680	Trp	Val	Trp	Ile	Val 685	Pro	Pro	Met		
TCC	GGA	AGC	ATC	ACC	CCT	GTG	TTC	CAC	CAG	GAG	ATG	CTC	AAC	TAC	CGG	2112	
Ser	Gly	Ser	Ile 690	Thr	Pro	Val 695	Phe	His	Gln	Glu	Met 700	Leu	Asn	Tyr	Arg		
CTC	ACC	CCC	TCC	TTC	GAA	TAC	CAG	CCT	GAT	CCC	TGG	AAC	ACG	CAT	GTC	2160	
Leu	Thr	Pro	Ser 705	Phe	Glu 710	Tyr	Gln	Pro	Asp 715	Pro	Trp	Asn	Thr	His 720	Val		
TGG	AAA	GGC	ACC	AAC	GGG	ACC	CCC	ACA	AAG	CGG	CGA	GCC	ATC	GGC	TTC	2208	
Trp	Lys	Gly	Thr 725	Asn	Gly	Thr	Pro	Thr 730	Lys	Arg	Arg	Ala	Ile	Gly 735	Phe		
AAG	AAG	CTA	GCA	GAA	GCT	GTC	AAG	TTC	TCG	GCC	AAG	CTG	ATG	GGG	CAG	2256	
Lys	Lys	Leu	Ala 740	Glu	Ala	Val	Lys 745	Phe	Ser	Ala	Lys	Leu	Met 750	Gly	Gln		

GCT	ATG	GCC	AAG	AGG	GTG	AAA	GCG	ACC	ATC	CTC	TAT	GCC	ACA	GAG	ACA	2304
Ala	Met	Ala	Lys	Arg	Val	Lys	Ala	Thr	Ile	Leu	Tyr	Ala	Thr	Glu	Thr	
		755					760					765				
GGC	AAA	TCG	CAA	GCT	TAT	GCC	AAG	ACC	TTG	TGT	GAG	ATC	TTC	AAA	CAC	2352
Gly	Lys	Ser	Gln	Ala	Tyr	Lys	Lys	Thr	Leu	Cys	Glu	Ile	Phe	Lys	His	
	770					775					780					
GCC	TTT	GAT	GCC	AAG	GTG	ATG	TCC	ATG	GAA	GAA	TAT	GAC	ATT	GTG	CAC	2400
Ala	Phe	Asp	Ala	Lys	Val	Met	Ser	Met	Glu	Glu	Tyr	Asp	Ile	Val	His	
	785				790					795					800	
CTG	GAA	CAT	GAA	ACT	CTG	GTC	CTT	GTG	GTC	ACC	AGC	ACC	TTT	GGC	AAT	2448
Leu	Glu	His	Glu	Thr	Leu	Val	Leu	Val	Val	Thr	Ser	Thr	Phe	Gly	Asn	
				805					810					815		
GGA	GAT	CCC	CCT	GAG	AAT	GGG	GAG	AAA	TTC	GGC	TGT	GCT	TTG	ATG	GAA	2496
Gly	Asp	Pro	Pro	Glu	Asn	Gly	Glu	Lys	Phe	Gly	Cys	Ala	Leu	Met	Glu	
			820					825					830			
ATG	AGG	CAC	CCC	AAC	TCT	GTG	CAG	GAA	GAA	AGG	AAG	AGC	TAC	AAG	GTC	2544
Met	Arg	His	Pro	Asn	Ser	Val	Gln	Glu	Glu	Arg	Lys	Ser	Tyr	Lys	Val	
		835					840					845				
CGA	TTC	AAC	AGC	GTC	TCC	TCC	TAC	TCT	GAC	TCC	CAA	AAA	TCA	TCA	GGC	2592
Arg	Phe	Asn	Ser	Val	Ser	Ser	Tyr	Ser	Asp	Ser	Gln	Lys	Ser	Ser	Gly	
	850					855					860					
GAT	GGG	CCC	GAC	CTC	AGA	GAC	AAC	TTT	GAG	AGT	GCT	GGA	CCC	CTG	GCC	2640
Asp	Gly	Pro	Asp	Leu	Arg	Asp	Asn	Phe	Glu	Ser	Ala	Gly	Pro	Leu	Ala	
	865				870					875					880	
AAT	GTG	AGG	TTC	TCA	GTT	TTT	GGC	CTC	GGC	TCA	CGA	GCA	TAC	CCT	CAC	2688
Asn	Val	Arg	Phe	Ser	Val	Phe	Gly	Leu	Gly	Ser	Arg	Ala	Tyr	Pro	His	
				885				890						895		
TTT	TGC	GCC	TTC	GGA	CAC	GCT	GTG	GAC	ACC	CTC	CTG	GAA	GAA	CTG	GGA	2736
Phe	Cys	Ala	Phe	Gly	His	Ala	Val	Asp	Thr	Leu	Leu	Glu	Glu	Leu	Gly	
			900					905					910			
GGG	GAG	AGG	ATC	CTG	AAG	ATG	AGG	GAA	GGG	GAT	GAG	CTC	TGT	GGG	CAG	2784
Gly	Glu	Arg	Ile	Leu	Lys	Met	Arg	Glu	Gly	Asp	Glu	Leu	Cys	Gly	Gln	
		915					920					925				
GAA	GAG	GCT	TTC	AGG	ACC	TGG	GCC	AAG	AAG	GTC	TTC	AAG	GCA	GCC	TGT	2832
Glu	Glu	Ala	Phe	Arg	Thr	Trp	Ala	Lys	Lys	Val	Phe	Lys	Ala	Ala	Cys	
	930					935					940					
GAT	GTC	TTC	TGT	GTG	GGA	GAT	GAT	GTC	AAC	ATT	GAA	AAG	GCC	AAC	AAT	2880
Asp	Val	Phe	Cys	Val	Gly	Asp	Asp	Val	Asn	Ile	Glu	Lys	Ala	Asn	Asn	
	945				950					955					960	
TCC	CTC	ATC	AGC	AAT	GAT	CGC	AGC	TGG	AAG	AGA	AAC	AAG	TTC	CGC	CTC	2928
Ser	Leu	Ile	Ser	Asn	Asp	Arg	Ser	Trp	Lys	Arg	Asn	Lys	Phe	Arg	Leu	
				965					970					975		
ACC	TTT	GTG	GCC	GAA	GCT	CCA	GAA	CTC	ACA	CAA	GGT	CTA	TCC	AAT	GTC	2976
Thr	Phe	Val	Ala	Glu	Ala	Pro	Glu	Leu	Thr	Gln	Gly	Leu	Ser	Asn	Val	
			980					985					990			
CAC	AAA	AAG	CGA	GTC	TCA	GCT	GCC	CGG	CTC	CTT	AGC	CGT	CAA	AAC	CTC	3024
His	Lys	Lys	Arg	Val	Ser	Ala	Ala	Arg	Leu	Leu	Ser	Arg	Gln	Asn	Leu	
		995					1000					1005				
CAG	AGC	CCT	AAA	TCC	AGT	CGG	TCA	ACT	ATC	TTC	GTG	CGT	CTC	CAC	ACC	3072
Gln	Ser	Pro	Lys	Ser	Ser	Arg	Ser	Thr	Ile	Phe	Val	Arg	Leu	His	Thr	
	1010					1015					1020					

AAC GGG AGC CAG GAG CTG CAG TAC CAG CCT GGG GAC CAC CTG GGT GTC Asn Gly Ser Gln Glu Leu Gln Tyr Gln Pro Gly Asp His Leu Gly Val 1025 1030 1035 1040	3120
TTC CCT GGC AAC CAC GAG GAC CTC GTG AAT GCC CTG ATC GAG CGG CTG Phe Pro Gly Asn His Glu Asp Leu Val Asn Ala Leu Ile Glu Arg Leu 1045 1050 1055	3168
GAG GAC GCG CCG CCT GTC AAC CAG ATG GTG AAA GTG GAA CTG CTG GAG Glu Asp Ala Pro Pro Val Asn Gln Met Val Lys Val Glu Leu Leu Glu 1060 1065 1070	3216
GAG CGG AAC ACG GCT TTA GGT GTC ATC AGT AAC TGG ACA GAC GAG CTC Glu Arg Asn Thr Ala Leu Gly Val Ile Ser Asn Trp Thr Asp Glu Leu 1075 1080 1085	3264
CGC CTC CCA CCC TGC ACC ATC TTC CAG GCC TTC AAG TAC TAC CTG GAC Arg Leu Pro Pro Cys Thr Ile Phe Gln Ala Phe Lys Tyr Tyr Leu Asp 1090 1095 1100	3312
ATC ACC ACG CCA CCA ACG CCC CTG CAG CTG CAG CAG TTT GCC TCC CTA Ile Thr Thr Pro Pro Thr Pro Leu Gln Leu Gln Gln Phe Ala Ser Leu 1105 1110 1115 1120	3360
GCT ACC AGC GAG AAG GAG AAG CAG CGT CTG CTG GTC CTC AGC AAG GGT Ala Thr Ser Glu Lys Glu Lys Gln Arg Leu Leu Val Leu Ser Lys Gly 1125 1130 1135	3408
TTG CAG GAG TAC GAG GAA TGG AAA TGG GGC AAG AAC CCC ACC ATC GTG Leu Gln Glu Tyr Glu Glu Trp Lys Trp Gly Lys Asn Pro Thr Ile Val 1140 1145 1150	3456
GAG GTG CTG GAG GAG TTC CCA TCT ATC CAG ATG CCG GCC ACC CTG CTC Glu Val Leu Glu Glu Phe Pro Ser Ile Gln Met Pro Ala Thr Leu Leu 1155 1160 1165	3504
CTG ACC CAG CTG TCC CTG CTG CAG CCC CGC TAC TAT TCC ATC AGC TCC Leu Thr Gln Leu Ser Leu Leu Gln Pro Arg Tyr Ser Ile Ser Ser 1170 1175 1180	3552
TCC CCA GAC ATG TAC CCT GAT GAA GTG CAC CTC ACT GTG GCC ATC GTT Ser Pro Asp Met Tyr Pro Asp Glu Val His Leu Thr Val Ala Ile Val 1185 1190 1195 1200	3600
TCC TAC CGC ACT CGA GAT GGA GAA GGA CCA ATT CAC CAC GGC GTA TGC Ser Tyr Arg Thr Arg Asp Gly Glu Gly Pro Ile His His Gly Val Cys 1205 1210 1215	3648
TCC TCC TGG CTC AAC CGG ATA CAG GCT GAC GAA CTG GTC CCC TGT TTC Ser Ser Trp Leu Asn Arg Ile Gln Ala Asp Glu Leu Val Pro Cys Phe 1220 1225 1230	3696
GTG AGA GGA GCA CCC AGC TTC CAC CTG CCC CGG AAC CCC CAA GTC CCC Val Arg Gly Ala Pro Ser Phe His Leu Pro Arg Asn Pro Gln Val Pro 1235 1240 1245	3744
TGC ATC CTC GTT GGA CCA GGC ACC GGC ATT GCC CCT TTC CGA AGC TTC Cys Ile Leu Val Gly Pro Gly Thr Gly Ile Ala Pro Phe Arg Ser Phe 1250 1255 1260	3792
TGG CAA CAG CGG CAA TTT GAT ATC CAA CAC AAA GGA ATG AAC CCC TGC Trp Gln Gln Arg Gln Phe Asp Ile Gln His Lys Gly Met Asn Pro Cys 1265 1270 1275 1280	3840
CCC ATG GTC CTG GTC TTC GGG TGC CGG CAA TCC AAG ATA GAT CAT ATC Pro Met Val Leu Val Phe Gly Cys Arg Gln Ser Lys Ile Asp His Ile 1285 1290 1295	3888

74

TAC AGG GAA GAG ACC CTG CAG GCC AAG AAC AAG GGG GTC TTC AGA GAG Tyr Arg Glu Glu Thr Leu Gln Ala Lys Asn Lys Gly Val Phe Arg Glu 1300 1305 1310	3936
CTG TAC ACG GCT TAC TCC CGG GAG CCA GAC AAA CCA AAG AAG TAC GTG Leu Tyr Thr Ala Tyr Ser Arg Glu Pro Asp Lys Pro Lys Lys Tyr Val 1315 1320 1325	3984
CAG GAC ATC CTG CAG GAG CAG CTG GCG GAG TCT GTG TAC CGA GCC CTG Gln Asp Ile Leu Gln Glu Gln Leu Ala Glu Ser Val Tyr Arg Ala Leu 1330 1335 1340	4032
AAG GAG CAA GGG GGC CAC ATA TAC GTC TGT GGG GAC GTC ACC ATG GCT Lys Glu Gln Gly Gly His Ile Tyr Val Cys Gly Asp Val Thr Met Ala 1345 1350 1355 1360	4080
GCT GAT GTC CTC AAA GCC ATC CAG CGC ATC ATG ACC CAG CAG GGG AAG Ala Asp Val Leu Lys Ala Ile Gln Arg Ile Met Thr Gln Gln Gly Lys 1365 1370 1375	4128
CTC TCG GCA GAG GAC GCC GGC GTA TTC ATC AGC CGG ATG AGG GAT GAC Leu Ser Ala Glu Asp Ala Gly Val Phe Ile Ser Arg Met Arg Asp Asp 1380 1385 1390	4176
AAC CGA TAC CAT GAG GAT ATT TTT GGA GTC ACC CTG CGA ACG TAC GAA Asn Arg Tyr His Glu Asp Ile Phe Gly Val Thr Leu Arg Thr Tyr Glu 1395 1400 1405	4224
GTG ACC AAC CGC CTT AGA TCT GAG TCC ATT GCC TTC ATT GAA GAG AGC Val Thr Asn Arg Leu Arg Ser Glu Ser Ile Ala Phe Ile Glu Glu Ser 1410 1415 1420	4272
AAA AAA GAC ACC GAT GAG GTT TTC AGC TCC TAACTGGACC CTCTTGCCCA Lys Lys Asp Thr Asp Glu Val Phe Ser Ser 1425 1430 143	4322
GCCGGCTGCA AGTTTGTAAG CGCGGGACAG A	4353

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1434 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Glu Asp His Met Phe Gly Val Gln Gln Ile Gln Pro Asn Val Ile 1 5 10 15
Ser Val Arg Leu Phe Lys Arg Lys Val Gly Gly Leu Gly Phe Leu Val 20 25 30
Lys Glu Arg Val Ser Lys Pro Pro Val Ile Ile Ser Asp Leu Ile Arg 35 40 45
Gly Gly Ala Ala Glu Gln Ser Gly Leu Ile Gln Ala Gly Asp Ile Ile 50 55 60
Leu Ala Val Asn Gly Arg Pro Leu Val Asp Leu Ser Tyr Asp Ser Ala 65 70 75 80
Leu Glu Val Leu Arg Gly Ile Ala Ser Glu Thr His Val Val Leu Ile 85 90 95

75

Leu Arg Gly Pro Glu Gly Phe Thr Thr His Leu Glu Thr Thr Phe Thr
 100 105 110
 Gly Asp Gly Thr Pro Lys Thr Ile Arg Val Thr Gln Pro Leu Gly Pro
 115 120 125
 Pro Thr Lys Ala Val Asp Leu Ser His Gln Pro Pro Ala Gly Lys Glu
 130 135 140
 Gln Pro Leu Ala Val Asp Gly Ala Ser Gly Pro Gly Asn Gly Pro Gln
 145 150 155 160
 His Ala Tyr Asp Asp Gly Gln Glu Ala Gly Ser Leu Pro His Ala Asn
 165 170 175
 Gly Leu Ala Pro Arg Pro Pro Gly Gln Asp Pro Ala Lys Lys Ala Thr
 180 185 190
 Arg Val Ser Leu Gln Gly Arg Gly Glu Asn Asn Glu Leu Leu Lys Glu
 195 200 205
 Ile Glu Pro Val Leu Ser Leu Leu Thr Ser Gly Ser Arg Gly Val Lys
 210 215 220
 Gly Gly Ala Pro Ala Lys Ala Glu Met Lys Asp Met Gly Ile Gln Val
 225 230 235 240
 Asp Arg Asp Leu Asp Gly Lys Ser His Lys Pro Leu Pro Leu Gly Val
 245 250 255
 Glu Asn Asp Arg Val Phe Asn Asp Leu Trp Gly Lys Gly Asn Val Pro
 260 265 270
 Val Val Leu Asn Asn Pro Tyr Ser Glu Lys Glu Gln Pro Pro Thr Ser
 275 280 285
 Gly Lys Gln Ser Pro Thr Lys Asn Gly Ser Pro Ser Lys Cys Pro Arg
 290 295 300
 Phe Leu Lys Val Lys Asn Trp Glu Thr Glu Val Val Leu Thr Asp Thr
 305 310 315 320
 Leu His Leu Lys Ser Thr Leu Glu Thr Gly Cys Thr Glu Tyr Ile Cys
 325 330 335
 Met Gly Ser Ile Met His Pro Ser Gln His Ala Arg Arg Pro Glu Asp
 340 345 350
 Val Arg Thr Lys Gly Gln Leu Phe Pro Leu Ala Lys Glu Phe Ile Asp
 355 360 365
 Gln Tyr Tyr Ser Ser Ile Lys Arg Phe Gly Ser Lys Ala His Met Glu
 370 375 380
 Arg Leu Glu Glu Val Asn Lys Glu Ile Asp Thr Thr Ser Thr Tyr Gln
 385 390 395 400
 Leu Lys Asp Thr Glu Leu Ile Tyr Gly Ala Lys His Ala Trp Arg Asn
 405 410 415
 Ala Ser Arg Cys Val Gly Arg Ile Gln Trp Ser Lys Leu Gln Val Phe
 420 425 430
 Asp Ala Arg Asp Cys Thr Thr Ala His Gly Met Phe Asn Tyr Ile Cys
 435 440 445
 Asn His Val Lys Tyr Ala Thr Asn Lys Gly Asn Leu Arg Ser Ala Ile

450					455					460					
Thr	Ile	Phe	Pro	Gln	Arg	Thr	Asp	Gly	Lys	His	Asp	Phe	Arg	Val	Trp
465					470					475				480	
Asn	Ser	Gln	Leu	Ile	Arg	Tyr	Ala	Gly	Tyr	Lys	Gln	Pro	Asp	Gly	Ser
				485					490					495	
Thr	Leu	Gly	Asp	Pro	Ala	Asn	Val	Gln	Phe	Thr	Glu	Ile	Cys	Ile	Gln
			500					505					510		
Gln	Gly	Trp	Lys	Pro	Pro	Arg	Gly	Arg	Phe	Asp	Val	Leu	Pro	Leu	Leu
		515					520					525			
Leu	Gln	Ala	Asn	Gly	Asn	Asp	Pro	Glu	Leu	Phe	Gln	Ile	Pro	Pro	Glu
		530				535					540				
Leu	Val	Leu	Glu	Val	Pro	Ile	Arg	His	Pro	Lys	Phe	Glu	Trp	Phe	Lys
545					550					555				560	
Asp	Leu	Gly	Leu	Lys	Trp	Tyr	Gly	Leu	Pro	Ala	Val	Ser	Asn	Met	Leu
				565					570				575		
Leu	Glu	Ile	Gly	Gly	Leu	Glu	Phe	Ser	Ala	Cys	Pro	Phe	Ser	Gly	Trp
			580					585					590		
Tyr	Met	Gly	Thr	Glu	Ile	Gly	Val	Arg	Asp	Tyr	Cys	Asp	Asn	Ser	Arg
		595					600					605			
Tyr	Asn	Ile	Leu	Glu	Glu	Val	Ala	Lys	Lys	Met	Asn	Leu	Asp	Met	Arg
	610					615					620				
Lys	Thr	Ser	Ser	Leu	Trp	Lys	Asp	Gln	Ala	Leu	Val	Glu	Ile	Asn	Ile
625					630				635					640	
Ala	Val	Leu	Tyr	Ser	Phe	Gln	Ser	Asp	Lys	Val	Thr	Ile	Val	Asp	His
				645					650				655		
His	Ser	Ala	Thr	Glu	Ser	Phe	Ile	Lys	His	Met	Glu	Asn	Glu	Tyr	Arg
			660					665					670		
Cys	Arg	Gly	Gly	Cys	Pro	Ala	Asp	Trp	Val	Trp	Ile	Val	Pro	Pro	Met
		675					680				685				
Ser	Gly	Ser	Ile	Thr	Pro	Val	Phe	His	Gln	Glu	Met	Leu	Asn	Tyr	Arg
	690					695				700					
Leu	Thr	Pro	Ser	Phe	Glu	Tyr	Gln	Pro	Asp	Pro	Trp	Asn	Thr	His	Val
705					710				715					720	
Trp	Lys	Gly	Thr	Asn	Gly	Thr	Pro	Thr	Lys	Arg	Arg	Ala	Ile	Gly	Phe
				725					730				735		
Lys	Lys	Leu	Ala	Glu	Ala	Val	Lys	Phe	Ser	Ala	Lys	Leu	Met	Gly	Gln
			740					745				750			
Ala	Met	Ala	Lys	Arg	Val	Lys	Ala	Thr	Ile	Leu	Tyr	Ala	Thr	Glu	Thr
		755				760					765				
Gly	Lys	Ser	Gln	Ala	Tyr	Ala	Lys	Thr	Leu	Cys	Glu	Ile	Phe	Lys	His
	770					775				780					
Ala	Phe	Asp	Ala	Lys	Val	Met	Ser	Met	Glu	Glu	Tyr	Asp	Ile	Val	His
785					790				795					800	
Leu	Glu	His	Glu	Thr	Leu	Val	Leu	Val	Val	Thr	Ser	Thr	Phe	Gly	Asn
				805					810				815		

Gly Asp Pro Pro Glu Asn Gly Glu Lys Phe Gly Cys Ala Leu Met Glu
 820 825 830
 Met Arg His Pro Asn Ser Val Gln Glu Glu Arg Lys Ser Tyr Lys Val
 835 840 845
 Arg Phe Asn Ser Val Ser Ser Tyr Ser Asp Ser Gln Lys Ser Ser Gly
 850 855 860
 Asp Gly Pro Asp Leu Arg Asp Asn Phe Glu Ser Ala Gly Pro Leu Ala
 865 870 875 880
 Asn Val Arg Phe Ser Val Phe Gly Leu Gly Ser Arg Ala Tyr Pro His
 885 890 895
 Phe Cys Ala Phe Gly His Ala Val Asp Thr Leu Leu Glu Glu Leu Gly
 900 905 910
 Gly Glu Arg Ile Leu Lys Met Arg Glu Gly Asp Glu Leu Cys Gly Gln
 915 920 925
 Glu Glu Ala Phe Arg Thr Trp Ala Lys Lys Val Phe Lys Ala Ala Cys
 930 935 940
 Asp Val Phe Cys Val Gly Asp Asp Val Asn Ile Glu Lys Ala Asn Asn
 945 950 955 960
 Ser Leu Ile Ser Asn Asp Arg Ser Trp Lys Arg Asn Lys Phe Arg Leu
 965 970 975
 Thr Phe Val Ala Glu Ala Pro Glu Leu Thr Gln Gly Leu Ser Asn Val
 980 985 990
 His Lys Lys Arg Val Ser Ala Ala Arg Leu Leu Ser Arg Gln Asn Leu
 995 1000 1005
 Gln Ser Pro Lys Ser Ser Arg Ser Thr Ile Phe Val Arg Leu His Thr
 1010 1015 1020
 Asn Gly Ser Gln Glu Leu Gln Tyr Gln Pro Gly Asp His Leu Gly Val
 1025 1030 1035 1040
 Phe Pro Gly Asn His Glu Asp Leu Val Asn Ala Leu Ile Glu Arg Leu
 1045 1050 1055
 Glu Asp Ala Pro Pro Val Asn Gln Met Val Lys Val Glu Leu Leu Glu
 1060 1065 1070
 Glu Arg Asn Thr Ala Leu Gly Val Ile Ser Asn Trp Thr Asp Glu Leu
 1075 1080 1085
 Arg Leu Pro Pro Cys Thr Ile Phe Gln Ala Phe Lys Tyr Tyr Leu Asp
 1090 1095 1100
 Ile Thr Thr Pro Pro Thr Pro Leu Gln Leu Gln Gln Phe Ala Ser Leu
 1105 1110 1115 1120
 Ala Thr Ser Glu Lys Glu Lys Gln Arg Leu Leu Val Leu Ser Lys Gly
 1125 1130 1135
 Leu Gln Glu Tyr Glu Glu Trp Lys Trp Gly Lys Asn Pro Thr Ile Val
 1140 1145 1150
 Glu Val Leu Glu Glu Phe Pro Ser Ile Gln Met Pro Ala Thr Leu Leu
 1155 1160 1165
 Leu Thr Gln Leu Ser Leu Leu Gln Pro Arg Tyr Tyr Ser Ile Ser Ser

1170	1175	1180
Ser Pro Asp Met Tyr	Pro Asp Glu Val His	Leu Thr Val Ala Ile Val
1185	1190	1195 1200
Ser Tyr Arg Thr	Arg Asp Gly Glu Gly	Pro Ile His His Gly Val Cys
	1205	1210 1215
Ser Ser Trp Leu Asn Arg	Ile Gln Ala Asp Glu	Leu Val Pro Cys Phe
	1220	1225 1230
Val Arg Gly Ala Pro Ser Phe	His Leu Pro Arg Asn	Pro Gln Val Pro
	1235	1240 1245
Cys Ile Leu Val Gly Pro Gly	Thr Gly Ile Ala Pro Phe	Arg Ser Phe
	1250	1255 1260
Trp Gln Gln Arg Gln Phe Asp	Ile Gln His Lys Gly	Met Asn Pro Cys
	1265	1270 1275 1280
Pro Met Val Leu Val Phe Gly	Cys Arg Gln Ser Lys	Ile Asp His Ile
	1285	1290 1295
Tyr Arg Glu Glu Thr Leu Gln	Ala Lys Asn Lys Gly	Val Phe Arg Glu
	1300	1305 1310
Leu Tyr Thr Ala Tyr Ser Arg	Glu Pro Asp Lys Pro	Lys Lys Tyr Val
	1315	1320 1325
Gln Asp Ile Leu Gln Glu Gln	Leu Ala Glu Ser Val	Tyr Arg Ala Leu
	1330	1335 1340
Lys Glu Gln Gly Gly His Ile	Tyr Val Cys Gly Asp	Val Thr Met Ala
	1345	1350 1355 1360
Ala Asp Val Leu Lys Ala Ile	Gln Arg Ile Met Thr	Gln Gln Gly Lys
	1365	1370 1375
Leu Ser Ala Glu Asp Ala Gly	Val Phe Ile Ser Arg	Met Arg Asp Asp
	1380	1385 1390
Asn Arg Tyr His Glu Asp Ile	Phe Gly Val Thr Leu	Arg Thr Tyr Glu
	1395	1400 1405
Val Thr Asn Arg Leu Arg Ser	Glu Ser Ile Ala Phe	Ile Glu Glu Ser
	1410	1415 1420
Lys Lys Asp Thr Asp Glu Val	Phe Ser Ser	
	1425	1430

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4780 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: Human NOS-SN gene, Nakane, et al,

FEBS Lett 316:175 (1993)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 431..4732

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GAGCGGACGG GCTCATGATG CCTCAGATCT GATCCGCATC TAACAGGCTG GCAATGAAGA	60
TACCCAGAGA ATAGTTCACA TCTATCATGC GTCACCTTCTA GACACAGCCA TCAGACGCAT	120
CTCCTCCCCCT TTCTGCCTGA CCTTAGGACA CGTCCCACCG CCTCTCTTGA CGTCTGCCTG	180
GTCAACCATC ACTTCCTTAG AGAATAAGGA GAGAGGCGGA TGCAGGAAAT CATGCCACCG	240
ACGGGCCACC AGCCATGAGT GGGTGACGCT GAGCTGACGT CAAAGACAGA GAGGGCTGAA	300
GCCTTGTCAG CACCTGTCAC CCCGGCTCCT GCTCTCCGTG TAGCCTGAAG CCTGGATCCT	360
CCTGGTGAAA TCATCTTGGC CTGATAGCAT TGTGAGGTCT TCAGACAGGA CCCCTCGGAA	420
GCTAGTTACC ATG GAG GAT CAC ATG TTC GGT GTT CAG CAA ATC CAG CCC	469
Met Glu Asp His Met Phe Gly Val Gln Ile Gln Pro	
1 5 10	
AAT GTC ATT TCT GTT CGT CTC TTC AAG CGC AAA GTT GGG GGC CTG GGA	517
Asn Val Ile Ser Val Arg Leu Phe Lys Arg Lys Val Gly Gly Leu Gly	
15 20 25	
TTT CTG GTG AAG GAG CGG GTC AGT AAG CCG CCC GTG ATC ATC TCT GAC	565
Phe Leu Val Lys Glu Arg Val Ser Lys Pro Pro Val Ile Ile Ser Asp	
30 35 40 45	
CTG ATT CGT GGG GGC GCC GCA GAG CAG AGT GGC CTC ATC CAG GCC GGA	613
Leu Ile Arg Gly Gly Ala Ala Glu Gln Ser Gly Leu Ile Gln Ala Gly	
50 55 60	
GAC ATC ATT CTT GCG GTC AAC GGC CGG CCC TTG GTG GAC CTG AGC TAT	661
Asp Ile Ile Leu Ala Val Asn Gly Arg Pro Leu Val Asp Leu Ser Tyr	
65 70 75	
GAC AGC GCC CTG GAG GTA CTC AGA GGC ATT GCC TCT GAG ACC CAC GTG	709
Asp Ser Ala Leu Glu Val Leu Arg Gly Ile Ala Ser Glu Thr His Val	
80 85 90	
GTC CTC ATT CTG AGG GGC CCT GAA GGT TTC ACC ACG CAC CTG GAG ACC	757
Val Leu Ile Leu Arg Gly Pro Glu Gly Phe Thr Thr His Leu Glu Thr	
95 100 105	
ACC TTT ACA GGT GAT GGG ACC CCC AAG ACC ATC CGG GTG ACA CAG CCC	805
Thr Phe Thr Gly Asp Gly Thr Pro Lys Thr Ile Arg Val Thr Gln Pro	
110 115 120 125	
CTG GGT CCC CCC ACC AAA GCC GTG GAT CTG TCC CAC CAG CCA CCG GCC	853
Leu Gly Pro Pro Thr Lys Ala Val Asp Leu Ser His Gln Pro Pro Ala	
130 135 140	
GGC AAA GAA CAG CCC CTG GCA GTG GAT GGG GCC TCG GGT CCC GGG AAT	901
Gly Lys Glu Gln Pro Leu Ala Val Asp Gly Ala Ser Gly Pro Gly Asn	
145 150 155	
GGG CCT CAG CAT GCC TAC GAT GAT GGG CAG GAG GCT GGC TCA CTC CCC	949
Gly Pro Gln His Ala Tyr Asp Asp Gly Gln Glu Ala Gly Ser Leu Pro	
160 165 170	

CAT	GCC	AAC	GGC	TGG	CCC	CAG	GCC	CCC	AGG	CAG	GAC	CCC	GCG	AAG	AAA	997
His	Ala	Asn	Gly	Trp	Pro	Gln	Ala	Pro	Arg	Gln	Asp	Pro	Ala	Lys	Lys	
	175					180					185					
GCA	ACC	AGA	GTC	AGC	CTC	CAA	GGC	AGA	GGG	GAG	AAC	AAT	GAA	CTG	CTC	1045
Ala	Thr	Arg	Val	Ser	Leu	Gln	Gly	Arg	Gly	Glu	Asn	Asn	Glu	Leu	Leu	
	190				195					200					205	
AAG	GAG	ATA	GAG	CCT	GTG	CTG	AGC	CTT	CTC	ACC	AGT	GGG	AGC	AGA	GGG	1093
Lys	Glu	Ile	Glu	Pro	Val	Leu	Ser	Leu	Leu	Thr	Ser	Gly	Ser	Arg	Gly	
				210					215					220		
GTC	AAG	GGA	GGG	GCA	CCT	GCC	AAG	GCA	GAG	ATG	AAA	GAT	ATG	GGA	ATC	1141
Val	Lys	Gly	Gly	Ala	Pro	Ala	Lys	Ala	Glu	Met	Lys	Asp	Met	Gly	Ile	
			225					230					235			
CAG	GTG	GAC	AGA	GAT	TTG	GAC	GGC	AAG	TCA	CAC	AAA	CCT	CTG	CCC	CTC	1189
Gln	Val	Asp	Arg	Asp	Leu	Asp	Gly	Lys	Ser	His	Lys	Pro	Leu	Pro	Leu	
		240					245					250				
GGC	GTG	GAG	AAC	GAC	CGA	GTG	TTC	AAT	GAC	CTA	TGG	GGG	AAG	GGC	AAT	1237
Gly	Val	Glu	Asn	Asp	Arg	Val	Phe	Asn	Asp	Leu	Trp	Gly	Lys	Gly	Asn	
	255					260					265					
GTG	CCT	GTC	GTC	CTC	AAC	AAC	CCA	TAT	TCA	GAG	AAG	GAG	CAG	CCC	CCC	1285
Val	Pro	Val	Val	Leu	Asn	Asn	Pro	Tyr	Ser	Glu	Lys	Glu	Gln	Pro	Pro	
	270				275					280					285	
ACC	TCA	GGA	AAA	CAG	TCC	CCC	ACA	AAG	AAT	GGC	AGC	CCC	TCC	AAG	TGT	1333
Thr	Ser	Gly	Lys	Gln	Ser	Pro	Thr	Lys	Asn	Gly	Ser	Pro	Ser	Lys	Cys	
				290					295					300		
CCA	CGC	TTC	CTC	AAG	GTC	AAG	AAC	TGG	GAG	ACT	GAG	GTG	GTT	CTC	ACT	1381
Pro	Arg	Phe	Leu	Lys	Val	Lys	Asn	Trp	Glu	Thr	Glu	Val	Val	Leu	Thr	
			305					310					315			
GAC	ACC	CTC	CAC	CTT	AAG	AGC	ACA	TTG	GAA	ACG	GGA	TGC	ACT	GAG	TAC	1429
Asp	Thr	Leu	His	Leu	Lys	Ser	Thr	Leu	Glu	Thr	Gly	Cys	Thr	Glu	Tyr	
		320					325					330				
ATC	TGC	ATG	GGC	TCC	ATC	ATG	CAT	CCT	TCT	CAG	CAT	GCA	AGG	AGG	CCT	1477
Ile	Cys	Met	Gly	Ser	Ile	Met	His	Pro	Ser	Gln	His	Ala	Arg	Arg	Pro	
	335					340					345					
GAA	GAC	GTC	CGC	ACA	AAA	GGA	CAG	CTC	TTC	CCT	CTC	GCC	AAA	GAG	TTT	1525
Glu	Asp	Val	Arg	Thr	Lys	Gly	Gln	Leu	Phe	Pro	Leu	Ala	Lys	Glu	Phe	
	350				355					360					365	
ATT	GAT	CAA	TAC	TAT	TCA	TCA	ATT	AAA	AGA	TTT	GGC	TCC	AAA	GCC	CAC	1573
Ile	Asp	Gln	Tyr	Tyr	Ser	Ser	Ile	Lys	Arg	Phe	Gly	Ser	Lys	Ala	His	
				370					375					380		
ATG	GAA	AGG	CTG	GAA	GAG	GTG	AAC	AAA	GAG	ATC	GAC	ACC	ACT	AGC	ACT	1621
Met	Glu	Arg	Leu	Glu	Glu	Val	Asn	Lys	Glu	Ile	Asp	Thr	Thr	Ser	Thr	
			385					390					395			
TAC	CAG	CTC	AAG	GAC	ACA	GAG	CTC	ATC	TAT	GGG	GCC	AAG	CAC	GCC	TGG	1669
Tyr	Gln	Leu	Lys	Asp	Thr	Glu	Leu	Ile	Tyr	Gly	Ala	Lys	His	Ala	Trp	
			400				405					410				
CGG	AAT	GCC	TCG	CGC	TGT	GTG	GGC	AGG	ATC	CAG	TGG	TCC	AAG	CTG	CAG	1717
Arg	Asn	Ala	Ser	Arg	Cys	Val	Gly	Arg	Ile	Gln	Trp	Ser	Lys	Leu	Gln	
	415					420					425					
GTA	TTC	GAT	GCC	CGT	GAC	TGC	ACC	ACG	GCC	CAC	GGG	ATG	TTC	AAC	TAC	1765
Val	Phe	Asp	Ala	Arg	Asp	Cys	Thr	Thr	Ala	His	Gly	Met	Phe	Asn	Tyr	
	430				435					440					445	

ATC	TGT	AAC	CAT	GTC	AAG	TAT	GCC	ACC	AAC	AAA	GGG	AAC	CTC	AGG	TCT	1813
Ile	Cys	Asn	His	Val	Lys	Tyr	Ala	Thr	Asn	Lys	Gly	Asn	Leu	Arg	Ser	
				450					455					460		
GCC	ATC	ACC	ATA	TTC	CCC	CAG	AGG	ACA	GAC	GGC	AAG	CAC	GAC	TTC	CGA	1861
Ala	Ile	Thr	Ile	Phe	Pro	Gln	Arg	Thr	Asp	Gly	Lys	His	Asp	Phe	Arg	
			465					470					475			
GTC	TGG	AAC	TCC	CAG	CTC	ATC	CGC	TAC	GCT	GGC	TAC	AAG	CAC	CGT	GAC	1909
Val	Trp	Asn	Ser	Gln	Leu	Ile	Arg	Tyr	Ala	Gly	Tyr	Lys	His	Arg	Asp	
		480					485					490				
GGC	TCC	ACC	CTG	GGG	GAC	CCA	GCC	AAT	GTG	CAG	TTC	ACA	GAG	ATA	TGC	1957
Gly	Ser	Thr	Leu	Gly	Asp	Pro	Ala	Asn	Val	Gln	Phe	Thr	Glu	Ile	Cys	
	495					500					505					
ATA	CAG	CAG	GGC	TGG	AAA	CCG	CCT	AGA	GGC	CGC	TTC	GAT	GTC	CTG	CCG	2005
Ile	Gln	Gln	Gly	Trp	Lys	Pro	Pro	Arg	Gly	Arg	Phe	Asp	Val	Leu	Pro	
510					515					520					525	
CTC	CTG	CTT	CAG	GCC	AAC	GGC	AAT	GAC	CCT	GAG	CTC	TTC	CAG	ATT	CCT	2053
Leu	Leu	Leu	Gln	Ala	Asn	Gly	Asn	Asp	Pro	Glu	Leu	Phe	Gln	Ile	Pro	
				530					535					540		
CCA	GAG	CTG	GTG	TTG	GAA	CTT	CCC	ATC	AGG	CAC	CCC	AAG	TTT	GAG	TGG	2101
Pro	Glu	Leu	Val	Leu	Glu	Leu	Pro	Ile	Arg	His	Pro	Lys	Phe	Glu	Trp	
			545					550					555			
TTC	AAG	GAC	CTG	GCG	CTG	AAG	TGG	TAC	GGC	CTC	CCC	GCC	GTG	TCC	AAC	2149
Phe	Lys	Asp	Leu	Ala	Leu	Lys	Trp	Tyr	Gly	Leu	Pro	Ala	Val	Ser	Asn	
		560					565					570				
ATG	CTC	CTA	GAG	ATT	GGC	GGC	CTG	GAG	TTC	AGC	GCC	TGT	CCC	TTC	AGT	2197
Met	Leu	Leu	Glu	Ile	Gly	Gly	Leu	Glu	Phe	Ser	Ala	Cys	Pro	Phe	Ser	
	575					580					585					
GGC	TGG	TAC	ATG	GGC	ACA	GAG	ATT	GGT	GTC	CGC	GAC	TAC	TGT	GAC	AAC	2245
Gly	Trp	Tyr	Met	Gly	Thr	Glu	Ile	Gly	Val	Arg	Asp	Tyr	Cys	Asp	Asn	
590					595					600					605	
TCC	CGC	TAC	AAT	ATC	CTG	GAG	GAA	GTG	GCC	AAG	AAG	ATG	AAC	TTA	GAC	2293
Ser	Arg	Tyr	Asn	Ile	Leu	Glu	Glu	Val	Ala	Lys	Lys	Met	Asn	Leu	Asp	
			610					615						620		
ATG	AGG	AAG	ACG	TCC	TCC	CTG	TGG	AAG	GAC	CAG	GCG	CTG	GTG	GAG	ATC	2341
Met	Arg	Lys	Thr	Ser	Ser	Leu	Trp	Lys	Asp	Gln	Ala	Leu	Val	Glu	Ile	
			625				630						635			
AAT	ATC	GCG	GTT	CTC	TAT	AGC	TTC	CAG	AGT	GAC	AAA	GTG	ACC	ATT	GTT	2389
Asn	Ile	Ala	Val	Leu	Tyr	Ser	Phe	Gln	Ser	Asp	Lys	Val	Thr	Ile	Val	
		640					645					650				
GAC	CAT	CAC	TCC	GCC	ACC	GAG	TCC	TTC	ATT	AAG	CAC	ATG	GAG	AAT	GAG	2437
Asp	His	His	Ser	Ala	Thr	Glu	Ser	Phe	Ile	Lys	His	Met	Glu	Asn	Glu	
		655				660					665					
TAC	CGC	TGC	CGG	GGG	GGC	TGC	CCT	GCC	GAC	TGG	GTG	TGG	ATC	GTG	CCC	2485
Tyr	Arg	Cys	Arg	Gly	Gly	Cys	Pro	Ala	Asp	Trp	Val	Trp	Ile	Val	Pro	
670					675					680					685	
CCC	ATG	TCC	GGA	AGC	ATC	ACC	CCT	GTG	TTC	CAC	CAG	GAG	ATG	CTC	AAC	2533
Pro	Met	Ser	Gly	Ser	Ile	Thr	Pro	Val	Phe	His	Gln	Glu	Met	Leu	Asn	
				690					695					700		
TAC	CGG	CTC	ACC	CCC	TCC	TTC	GAA	TAC	CAG	CCT	GAT	CCC	TGG	AAC	ACG	2581
Tyr	Arg	Leu	Thr	Pro	Ser	Phe	Glu	Tyr	Gln	Pro	Asp	Pro	Trp	Asn	Thr	
			705					710					715			

CAT	GTC	TGG	AAA	GGC	ACC	AAC	GGG	ACC	CCC	ACA	AAG	CGG	CGA	GCC	ATC	2629
His	Val	Trp	Lys	Gly	Thr	Asn	Gly	Thr	Pro	Thr	Lys	Arg	Arg	Ala	Ile	
		720					725					730				
GGC	TTC	AAG	AAG	CTA	GCA	GAA	GCT	GTC	AAG	TTC	TCG	GCC	AAG	CTG	ATG	2677
Gly	Phe	Lys	Lys	Leu	Ala	Glu	Ala	Val	Lys	Phe	Ser	Ala	Lys	Leu	Met	
		735				740					745					
GGG	CAG	GCT	ATG	GCC	AAG	AGG	GTG	AAA	GCG	ACC	ATC	CTC	TAT	GCC	ACA	2725
Gly	Gln	Ala	Met	Ala	Lys	Arg	Val	Lys	Ala	Thr	Ile	Leu	Tyr	Ala	Thr	
		750			755					760					765	
GAG	ACA	GGC	AAA	TCG	CAA	GCT	TAT	GCC	AAG	ACC	TTG	TGT	GAG	ATC	TTC	2773
Glu	Thr	Gly	Lys	Ser	Gln	Ala	Tyr	Ala	Lys	Thr	Leu	Cys	Glu	Ile	Phe	
				770					775					780		
AAA	CAC	GCC	TTT	GAT	GCC	AAG	GTG	ATG	TCC	ATG	GAA	GAA	TAT	GAC	ATT	2821
Lys	His	Ala	Phe	Asp	Ala	Lys	Val	Met	Ser	Met	Glu	Glu	Tyr	Asp	Ile	
			785					790					795			
GTG	CAC	CTG	GAA	CAT	GAA	ACT	CTG	GTC	CTT	GTG	GTC	ACC	AGC	ACC	TTT	2869
Val	His	Leu	Glu	His	Glu	Thr	Leu	Val	Leu	Val	Val	Thr	Ser	Thr	Phe	
		800					805					810				
GGC	AAT	GGA	GAT	CCC	CCT	GAG	AAT	GGG	GAG	AAA	TTC	GGC	TGT	GCT	TTG	2917
Gly	Asn	Gly	Asp	Pro	Pro	Glu	Asn	Gly	Glu	Lys	Phe	Gly	Cys	Ala	Leu	
		815				820					825					
ATG	GAA	ATG	AGG	CAC	CCC	AAC	TCT	GTG	CAG	GAA	GAA	AGG	AAG	AGC	TAC	2965
Met	Glu	Met	Arg	His	Pro	Asn	Ser	Val	Gln	Glu	Glu	Arg	Lys	Ser	Tyr	
		830			835					840					845	
AAG	GTC	CGA	TTC	AAC	AGC	GTC	TCC	TCC	TAC	TCT	GAC	TCC	CAA	AAA	TCA	3013
Lys	Val	Arg	Phe	Asn	Ser	Val	Ser	Ser	Tyr	Ser	Asp	Ser	Gln	Lys	Ser	
				850					855					860		
TCA	GGC	GAT	GGG	CCC	GAC	CTC	AGA	GAC	AAC	TTT	GAG	AGT	GCT	GGA	CCC	3061
Ser	Gly	Asp		Pro	Asp	Leu	Arg	Asp	Asn	Phe	Glu	Ser	Ala	Gly	Pro	
			865					870					875			
CTG	GCC	AAT	GTG	AGG	TTC	TCA	GTT	TTT	GGC	CTC	GGC	TCA	CGA	GCA	TAC	3109
Leu	Ala	Asn	Val	Arg	Phe	Ser	Val	Phe	Gly	Leu	Gly	Ser	Arg	Ala	Tyr	
		880					885					890				
CCT	CAC	TTT	TGC	GCC	TTC	GGA	CAC	GCT	GTG	GAC	ACC	CTC	CTG	GAA	GAA	3157
Pro	His	Phe	Cys	Ala	Phe	Gly	His	Ala	Val	Asp	Thr	Leu	Leu	Glu	Glu	
		895				900					905					
CTG	GGA	GGG	GAG	AGG	ATC	CTG	AAG	ATG	AGG	GAA	GGG	GAT	GAG	CTC	TGT	3205
Leu	Gly	Gly	Glu	Arg	Ile	Leu	Lys	Met	Arg	Glu	Gly	Asp	Glu	Leu	Cys	
		910			915					920					925	
GGG	CAG	GAA	GAG	GCT	TTC	AGG	ACC	TGG	GCC	AAG	AAG	GTC	TTC	AAG	GCA	3253
Gly	Gln	Glu	Glu	Ala	Phe	Arg	Thr	Trp	Ala	Lys	Lys	Val	Phe	Lys	Ala	
				930					935					940		
GCC	TGT	GAT	GTC	TTC	TGT	GTG	GGA	GAT	GAT	GTC	AAC	ATT	GAA	AAG	GCC	3301
Ala	Cys	Asp	Val	Phe	Cys	Val	Gly	Asp	Asp	Val	Asn	Ile	Glu	Lys	Ala	
			945					950					955			
AAC	AAT	TCC	CTC	ATC	AGC	AAT	GAT	CGC	AGC	TGG	AAG	AGA	AAC	AAG	TTC	3349
Asn	Asn	Ser	Leu	Ile	Ser	Asn	Asp	Arg	Ser	Trp	Lys	Arg	Asn	Lys	Phe	
		960					965					970				
CGC	CTC	ACC	TTT	GTG	GCC	GAA	GCT	CCA	GAA	CTC	ACA	CAA	GGT	CTA	TCC	3397
Arg	Leu	Thr	Phe	Val	Ala	Glu	Ala	Pro	Glu	Leu	Thr	Gln	Gly	Leu	Ser	
		975				980					985					

AAT GTC CAC AAA AAG CGA GTC TCA GCT GCC CGG CTC CTT AGC CGT CAA Asn Val His Lys Lys Arg Val Ser Ala Ala Arg Leu Leu Ser Arg Gln 990 995 1000 1005	3445
AAC CTC CAG AGC CCT AAA TCC AGT CGG TCA ACT ATC TTC GTG CGT CTC Asn Leu Gln Ser Pro Lys Ser Ser Arg Ser Thr Ile Phe Val Arg Leu 1010 1015 1020	3493
CAC ACC AAC GGG AGC CAG GAG CTG CAG TAC CAG CCT GGG GAC CAC CTG His Thr Asn Gly Ser Gln Glu Leu Gln Tyr Gln Pro Gly Asp His Leu 1025 1030 1035	3541
GGT GTC TTC CCT GGC AAC CAC GAG GAC CTC GTG AAT GCC CTG ATC GAG Gly Val Phe Pro Gly Asn His Glu Asp Leu Val Asn Ala Leu Ile Glu 1040 1045 1050	3589
CGG CTG GAG GAC GCG CCG CCT GTC AAC CAG ATG GTG AAA GTG GAA CTG Arg Leu Glu Asp Ala Pro Pro Val Asn Gln Met Val Lys Val Glu Leu 1055 1060 1065	3637
CTG GAG GAG CGG AAC ACG GCT TTA GGT GTC ATC AGT AAC TGG ACA GAC Leu Glu Glu Arg Asn Thr Ala Leu Gly Val Ile Ser Asn Trp Thr Asp 1070 1075 1080 1085	3685
GAG CTC CGC CTC CCG CCC TGC ACC ATC TTC CAG GCC TTC AAG TAC TAC Glu Leu Arg Leu Pro Pro Cys Thr Ile Phe Gln Ala Phe Lys Tyr Tyr 1090 1095 1100	3733
CTG GAC ATC ACC ACG CCA CCA ACG CCT CTG CAG CTG CAG CAG TTT GCC Leu Asp Ile Thr Thr Pro Pro Thr Pro Leu Gln Leu Gln Gln Phe Ala 1105 1110 1115	3781
TCC CTA GCT ACC AGC GAG AAG GAG AAG CAG CGT CTG CTG GTC CTC AGC Ser Leu Ala Thr Ser Glu Lys Glu Lys Gln Arg Leu Leu Val Leu Ser 1120 1125 1130	3829
AAG GGT TTG CAG GAG TAC GAG GAA TGG AAA TGG GGC AAG AAC CCC ACC Lys Gly Leu Gln Glu Tyr Glu Glu Trp Lys Trp Gly Lys Asn Pro Thr 1135 1140 1145	3877
ATC GTG GAG GTG CTG GAG GAG TTC CCA TCT ATC CAG ATG CCG GCC ACC Ile Val Glu Val Leu Glu Glu Phe Pro Ser Ile Gln Met Pro Ala Thr 1150 1155 1160 1165	3925
CTG CTC CTG ACC CAG CTG TCC CTG CTG CAG CCC CGC TAC TAT TCC ATC Leu Leu Leu Thr Gln Leu Ser Leu Leu Gln Pro Arg Tyr Tyr Ser Ile 1170 1175 1180	3973
AGC TCC TCC CCA GAC ATG TAC CCT GAT GAA GTG CAC CTC ACT GTG GCC Ser Ser Ser Pro Asp Met Tyr Pro Asp Glu Val His Leu Thr Val Ala 1185 1190 1195	4021
ATC GTT TCC TAC CGC ACT CGA GAT GGA GAA GGA CCA ATT CAC CAC GGC Ile Val Ser Tyr Arg Thr Arg Asp Gly Glu Gly Pro Ile His His Gly 1200 1205 1210	4069
GTA TGC TCC TCC TGG CTC AAC CGG ATA CAG GCT GAC GAA CTG GTC CCC Val Cys Ser Ser Trp Leu Asn Arg Ile Gln Ala Asp Glu Leu Val Pro 1215 1220 1225	4117
TGT TTC GTG AGA GGA GCA CCC AGC TTC CAC CTG CCC CGG AAC CCC CAA Cys Phe Val Arg Gly Ala Pro Ser Phe His Leu Pro Arg Asn Pro Gln 1230 1235 1240 1245	4165
GTC CCC TGC ATC CTC GTT GGA CCA GGC ACC GGC ATT GCC CCT TTC CGA Val Pro Cys Ile Leu Val Gly Pro Gly Thr Gly Ile Ala Pro Phe Arg 1250 1255 1260	4213

84

AGC TTC TGG CAA CAG CGG CAA TTT GAT ATC CAA CAC AAA GGA ATG AAC	4261
Ser Phe Trp Gln Gln Arg Gln Phe Asp Ile Gln His Lys Gly Met Asn	
1265 1270 1275	
CCC TGC CCC ATG GTC CTG GTC TTC GGG TGC CGG CAA TCC AAG ATA GAT	4309
Pro Cys Pro Met Val Leu Val Phe Gly Cys Arg Gln Ser Lys Ile Asp	
1280 1285 1290	
CAT ATC TAC AGG GAA GAG ACC CTG CAG GCC AAG AAC AAG GGG GTC TTC	4357
His Ile Tyr Arg Glu Glu Thr Leu Gln Ala Lys Asn Lys Gly Val Phe	
1295 1300 1305	
AGA GAG CTG TAC ACG GCT TAC TCC CGG GAG CCA GAC AAA CCA AAG AAG	4405
Arg Glu Leu Tyr Thr Ala Tyr Ser Arg Glu Pro Asp Lys Pro Lys Lys	
1310 1315 1320 1325	
TAC GTG CAG GAC ATC CTG CAG GAG CAG CTG GCG GAG TCT GTG TAC CGA	4453
Tyr Val Gln Asp Ile Leu Gln Glu Gln Leu Ala Glu Ser Val Tyr Arg	
1330 1335 1340	
GCC CTG AAG GAG CAA GGG GGC CAC ATA TAC GTC TGT GGG GAC GTC ACC	4501
Ala Leu Lys Glu Gln Gly Gly His Ile Tyr Val Cys Gly Asp Val Thr	
1345 1350 1355	
ATG GCT GCT GAT GTC CTC AAA GCC ATC CAG CGC ATC ATG ACC CAG CAG	4549
Met Ala Ala Asp Val Leu Lys Ala Ile Gln Arg Ile Met Thr Gln Gln	
1360 1365 1370	
GGG AAG CTC TCG GCA GAG GAC GCC GGC GTA TTC ATC AGC CGG ATG AGG	4597
Gly Lys Leu Ser Ala Glu Asp Ala Gly Val Phe Ile Ser Arg Met Arg	
1375 1380 1385	
GAT GAC AAC CGA TAC CAT GAG GAT ATT TTT GGA GTC ACC CTG CGA ACG	4645
Asp Asp Asn Arg Tyr His Glu Asp Ile Phe Gly Val Thr Leu Arg Thr	
1390 1395 1400 1405	
ATC GAA GTG ACC AAC CGC CTT AGA TCT GAG TCC ATT GCC TTC ATT GAA	4693
Ile Glu Val Thr Asn Arg Leu Arg Ser Glu Ser Ile Ala Phe Ile Glu	
1410 1415 1420	
GAG AGC AAA AAA GAC ACC GAT GAG GTT TTC AGC TCC TAACTGGACC	4739
Glu Ser Lys Lys Asp Thr Asp Glu Val Phe Ser Ser	
1425 1430	
CTCTTGCCCA GCCGGCTGCA AGTTTGTAAG CGCGGGACAG A	4780

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1433 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Glu Asp His Met Phe Gly Val Gln Gln Ile Gln Pro Asn Val Ile
 1 5 10 15

Ser Val Arg Leu Phe Lys Arg Lys Val Gly Gly Leu Gly Phe Leu Val
 20 25 30

Lys Glu Arg Val Ser Lys Pro Pro Val Ile Ile Ser Asp Leu Ile Arg
 35 40 45

85

Gly Gly Ala Ala Glu Gln Ser Gly Leu Ile Gln Ala Gly Asp Ile Ile
 50 55 60
 Leu Ala Val Asn Gly Arg Pro Leu Val Asp Leu Ser Tyr Asp Ser Ala
 65 70 75 80
 Leu Glu Val Leu Arg Gly Ile Ala Ser Glu Thr His Val Val Leu Ile
 85 90 95
 Leu Arg Gly Pro Glu Gly Phe Thr Thr His Leu Glu Thr Thr Phe Thr
 100 105 110
 Gly Asp Gly Thr Pro Lys Thr Ile Arg Val Thr Gln Pro Leu Gly Pro
 115 120 125
 Pro Thr Lys Ala Val Asp Leu Ser His Gln Pro Pro Ala Gly Lys Glu
 130 135 140
 Gln Pro Leu Ala Val Asp Gly Ala Ser Gly Pro Gly Asn Gly Pro Gln
 145 150 155 160
 His Ala Tyr Asp Asp Gly Gln Glu Ala Gly Ser Leu Pro His Ala Asn
 165 170 175
 Gly Trp Pro Gln Ala Pro Arg Gln Asp Pro Ala Lys Lys Ala Thr Arg
 180 185 190
 Val Ser Leu Gln Gly Arg Gly Glu Asn Asn Glu Leu Leu Lys Glu Ile
 195 200 205
 Glu Pro Val Leu Ser Leu Leu Thr Ser Gly Ser Arg Gly Val Lys Gly
 210 215 220
 Gly Ala Pro Ala Lys Ala Glu Met Lys Asp Met Gly Ile Gln Val Asp
 225 230 235 240
 Arg Asp Leu Asp Gly Lys Ser His Lys Pro Leu Pro Leu Gly Val Glu
 245 250 255
 Asn Asp Arg Val Phe Asn Asp Leu Trp Gly Lys Gly Asn Val Pro Val
 260 265 270
 Val Leu Asn Asn Pro Tyr Ser Glu Lys Glu Gln Pro Pro Thr Ser Gly
 275 280 285
 Lys Gln Ser Pro Thr Lys Asn Gly Ser Pro Ser Lys Cys Pro Arg Phe
 290 295 300
 Leu Lys Val Lys Asn Trp Glu Thr Glu Val Val Leu Thr Asp Thr Leu
 305 310 315 320
 His Leu Lys Ser Thr Leu Glu Thr Gly Cys Thr Glu Tyr Ile Cys Met
 325 330 335
 Gly Ser Ile Met His Pro Ser Gln His Ala Arg Arg Pro Glu Asp Val
 340 345 350
 Arg Thr Lys Gly Gln Leu Phe Pro Leu Ala Lys Glu Phe Ile Asp Gln
 355 360 365
 Tyr Tyr Ser Ser Ile Lys Arg Phe Gly Ser Lys Ala His Met Glu Arg
 370 375 380
 Leu Glu Glu Val Asn Lys Glu Ile Asp Thr Thr Ser Thr Tyr Gln Leu
 385 390 395 400
 Lys Asp Thr Glu Leu Ile Tyr Gly Ala Lys His Ala Trp Arg Asn Ala

86

405					410					415					
Ser	Arg	Cys	Val	Gly	Arg	Ile	Gln	Trp	Ser	Lys	Leu	Gln	Val	Phe	Asp
			420					425					430		
Ala	Arg	Asp	Cys	Thr	Thr	Ala	His	Gly	Met	Phe	Asn	Tyr	Ile	Cys	Asn
		435					440					445			
His	Val	Lys	Tyr	Ala	Thr	Asn	Lys	Gly	Asn	Leu	Arg	Ser	Ala	Ile	Thr
	450					455					460				
Ile	Phe	Pro	Gln	Arg	Thr	Asp	Gly	Lys	His	Asp	Phe	Arg	Val	Trp	Asn
465					470				475					480	
Ser	Gln	Leu	Ile	Arg	Tyr	Ala	Gly	Tyr	Lys	His	Arg	Asp	Gly	Ser	Thr
				485					490					495	
Leu	Gly	Asp	Pro	Ala	Asn	Val	Gln	Phe	Thr	Glu	Ile	Cys	Ile	Gln	Gln
			500					505					510		
Gly	Trp	Lys	Pro	Pro	Arg	Gly	Arg	Phe	Asp	Val	Leu	Pro	Leu	Leu	Leu
		515					520					525			
Gln	Ala	Asn	Gly	Asn	Asp	Pro	Glu	Leu	Phe	Gln	Ile	Pro	Pro	Glu	Leu
	530					535					540				
Val	Leu	Glu	Leu	Pro	Ile	Arg	His	Pro	Lys	Phe	Glu	Trp	Phe	Lys	Asp
545					550					555					560
Leu	Ala	Leu	Lys	Trp	Tyr	Gly	Leu	Pro	Ala	Val	Ser	Asn	Met	Leu	Leu
				565					570					575	
Glu	Ile	Gly	Gly	Leu	Glu	Phe	Ser	Ala	Cys	Pro	Phe	Ser	Gly	Trp	Tyr
			580					585					590		
Met	Gly	Thr	Glu	Ile	Gly	Val	Arg	Asp	Tyr	Cys	Asp	Asn	Ser	Arg	Tyr
		595					600					605			
Asn	Ile	Leu	Glu	Glu	Val	Ala	Lys	Lys	Met	Asn	Leu	Asp	Met	Arg	Lys
	610					615					620				
Thr	Ser	Ser	Leu	Trp	Lys	Asp	Gln	Ala	Leu	Val	Glu	Ile	Asn	Ile	Ala
625					630					635					640
Val	Leu	Tyr	Ser	Phe	Gln	Ser	Asp	Lys	Val	Thr	Ile	Val	Asp	His	His
				645					650					655	
Ser	Ala	Thr	Glu	Ser	Phe	Ile	Lys	His	Met	Glu	Asn	Glu	Tyr	Arg	Cys
			660					665					670		
Arg	Gly	Gly	Cys	Pro	Ala	Asp	Trp	Val	Trp	Ile	Val	Pro	Pro	Met	Ser
		675					680					685			
Gly	Ser	Ile	Thr	Pro	Val	Phe	His	Gln	Glu	Met	Leu	Asn	Tyr	Arg	Leu
	690					695					700				
Thr	Pro	Ser	Phe	Glu	Tyr	Gln	Pro	Asp	Pro	Trp	Asn	Thr	His	Val	Trp
705					710					715					720
Lys	Gly	Thr	Asn	Gly	Thr	Pro	Thr	Lys	Arg	Arg	Ala	Ile	Gly	Phe	Lys
				725					730					735	
Lys	Leu	Ala	Glu	Ala	Val	Lys	Phe	Ser	Ala	Lys	Leu	Met	Gly	Gln	Ala
			740					745					750		
Met	Ala	Lys	Arg	Val	Lys	Ala	Thr	Ile	Leu	Tyr	Ala	Thr	Glu	Thr	Gly
		755					760					765			

Lys Ser Gln Ala Tyr Ala Lys Thr Leu Cys Glu Ile Phe Lys His Ala
 770 775 780
 Phe Asp Ala Lys Val Met Ser Met Glu Glu Tyr Asp Ile Val His Leu
 785 790 795 800
 Glu His Glu Thr Leu Val Leu Val Val Thr Ser Thr Phe Gly Asn Gly
 805 810 815
 Asp Pro Pro Glu Asn Gly Glu Lys Phe Gly Cys Ala Leu Met Glu Met
 820 825 830
 Arg His Pro Asn Ser Val Gln Glu Glu Arg Lys Ser Tyr Lys Val Arg
 835 840 845
 Phe Asn Ser Val Ser Ser Tyr Ser Asp Ser Gln Lys Ser Ser Gly Asp
 850 855 860
 Gly Pro Asp Leu Arg Asp Asn Phe Glu Ser Ala Gly Pro Leu Ala Asn
 865 870 875 880
 Val Arg Phe Ser Val Phe Gly Leu Gly Ser Arg Ala Tyr Pro His Phe
 885 890 895
 Cys Ala Phe Gly His Ala Val Asp Thr Leu Leu Glu Glu Leu Gly Gly
 900 905 910
 Glu Arg Ile Leu Lys Met Arg Glu Gly Asp Glu Leu Cys Gly Gln Glu
 915 920 925
 Glu Ala Phe Arg Thr Trp Ala Lys Lys Val Phe Lys Ala Ala Cys Asp
 930 935 940
 Val Phe Cys Val Gly Asp Asp Val Asn Ile Glu Lys Ala Asn Asn Ser
 945 950 955 960
 Leu Ile Ser Asn Asp Arg Ser Trp Lys Arg Asn Lys Phe Arg Leu Thr
 965 970 975
 Phe Val Ala Glu Ala Pro Glu Leu Thr Gln Gly Leu Ser Asn Val His
 980 985 990
 Lys Lys Arg Val Ser Ala Ala Arg Leu Leu Ser Arg Gln Asn Leu Gln
 995 1000 1005
 Ser Pro Lys Ser Ser Arg Ser Thr Ile Phe Val Arg Leu His Thr Asn
 1010 1015 1020
 Gly Ser Gln Glu Leu Gln Tyr Gln Pro Gly Asp His Leu Gly Val Phe
 1025 1030 1035 1040
 Pro Gly Asn His Glu Asp Leu Val Asn Ala Leu Ile Glu Arg Leu Glu
 1045 1050 1055
 Asp Ala Pro Pro Val Asn Gln Met Val Lys Val Glu Leu Leu Glu Glu
 1060 1065 1070
 Arg Asn Thr Ala Leu Gly Val Ile Ser Asn Trp Thr Asp Glu Leu Arg
 1075 1080 1085
 Leu Pro Pro Cys Thr Ile Phe Gln Ala Phe Lys Tyr Tyr Leu Asp Ile
 1090 1095 1100
 Thr Thr Pro Pro Thr Pro Leu Gln Leu Gln Gln Phe Ala Ser Leu Ala
 1105 1110 1115 1120
 Thr Ser Glu Lys Glu Lys Gln Arg Leu Leu Val Leu Ser Lys Gly Leu

88

1125	1130	1135
Gln Glu Tyr Glu Glu Trp Lys Trp Gly Lys Asn Pro Thr Ile Val Glu 1140	1145	1150
Val Leu Glu Glu Phe Pro Ser Ile Gln Met Pro Ala Thr Leu Leu Leu 1155	1160	1165
Thr Gln Leu Ser Leu Leu Gln Pro Arg Tyr Tyr Ser Ile Ser Ser Ser 1170	1175	1180
Pro Asp Met Tyr Pro Asp Glu Val His Leu Thr Val Ala Ile Val Ser 1185	1190	1195
Tyr Arg Thr Arg Asp Gly Glu Gly Pro Ile His His Gly Val Cys Ser 1205	1210	1215
Ser Trp Leu Asn Arg Ile Gln Ala Asp Glu Leu Val Pro Cys Phe Val 1220	1225	1230
Arg Gly Ala Pro Ser Phe His Leu Pro Arg Asn Pro Gln Val Pro Cys 1235	1240	1245
Ile Leu Val Gly Pro Gly Thr Gly Ile Ala Pro Phe Arg Ser Phe Trp 1250	1255	1260
Gln Gln Arg Gln Phe Asp Ile Gln His Lys Gly Met Asn Pro Cys Pro 1265	1270	1275
Met Val Leu Val Phe Gly Cys Arg Gln Ser Lys Ile Asp His Ile Tyr 1285	1290	1295
Arg Glu Glu Thr Leu Gln Ala Lys Asn Lys Gly Val Phe Arg Glu Leu 1300	1305	1310
Tyr Thr Ala Tyr Ser Arg Glu Pro Asp Lys Pro Lys Lys Tyr Val Gln 1315	1320	1325
Asp Ile Leu Gln Glu Gln Leu Ala Glu Ser Val Tyr Arg Ala Leu Lys 1330	1335	1340
Glu Gln Gly Gly His Ile Tyr Val Cys Gly Asp Val Thr Met Ala Ala 1345	1350	1355
Asp Val Leu Lys Ala Ile Gln Arg Ile Met Thr Gln Gln Gly Lys Leu 1365	1370	1375
Ser Ala Glu Asp Ala Gly Val Phe Ile Ser Arg Met Arg Asp Asp Asn 1380	1385	1390
Arg Tyr His Glu Asp Ile Phe Gly Val Thr Leu Arg Thr Ile Glu Val 1395	1400	1405
Thr Asn Arg Leu Arg Ser Glu Ser Ile Ala Phe Ile Glu Glu Ser Lys 1410	1415	1420
Lys Asp Thr Asp Glu Val Phe Ser Ser 1425	1430	

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 256 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: EPO-1 HRE element

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GAAGTGAAC CACCAATATG ACTCTGGCT TTTCTGTTTT CTGGGAACCT CCAAATCCCC	60
TGGCTCTGTC CCACTCCTGG CAGCAGTGCA GCAGGTCCAG GTCCGGGAAA TGAGGGGTGG	120
AGGGGGCTGG GCCCTACGTG CTGTCTACA CAGCCTGTCT GACCTCTCGA CCTACCGGCC	180
TAGGCCACAA GCTCTGCCTA CGCTGGTCAA TAAGGTGTCT CCATTCAAGG CCTCACCGCA	240
GTAAGGCAGC TGCCAA	256

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 42 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: 42 bp EPO 3' hypoxia response enhancer element (Madan, et al, PNAS 90:3928, 1993)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGGCCCTACG TGCTGTCTCA CACAGCCTGT CTGACCTCTC GA	42
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(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 86 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (C) INDIVIDUAL ISOLATE: 86 nucleotide fragment from α MHC promoter

TAC	ACT	GAA	GAT	GGT	AAC	TGG	GAT	CTT	GTG	GGA	AAC	AAC	ACC	CCT	ATT	543
Tyr	Thr	Glu	Asp	Gly	Asn	Trp	Asp	Leu	Val	Gly	Asn	Asn	Thr	Pro	Ile	
		140						145					150			
TTC	TTC	ATC	AGG	GAT	GCC	ATA	TTG	TTT	CCA	TCC	TTT	ATC	CAT	AGC	CAG	591
Phe	Phe	Ile	Arg	Asp	Ala	Ile	Leu	Phe	Pro	Ser	Phe	Ile	His	Ser	Gln	
		155					160					165				
AAG	AGA	AAC	CCA	CAG	ACT	CAC	CTG	AAG	GAT	CCT	GAC	ATG	GTC	TGG	GAC	639
Lys	Arg	Asn	Pro	Gln	Thr	His	Leu	Lys	Asp	Pro	Asp	Met	Val	Trp	Asp	
	170					175					180					
TTC	TGG	AGT	CTT	CGT	CCC	GAG	TCT	CTC	CAT	CAG	GTT	TCT	TTC	TTG	TTC	687
Phe	Trp	Ser	Leu	Arg	Pro	Glu	Ser	Leu	His	Gln	Val	Ser	Phe	Leu	Phe	
185					190					195				200		
AGT	GAC	CGA	GGG	ATT	CCC	GAT	GGT	CAC	CGG	CAC	ATG	AAT	GGC	TAT	GGA	735
Ser	Asp	Arg	Gly	Ile	Pro	Asp	Gly	His	Arg	His	Met	Asn	Gly	Tyr	Gly	
				205					210					215		
TCA	CAC	ACC	TTC	AAG	TTG	GTT	AAT	GCA	GAT	GGA	GAG	GCA	GTC	TAT	TGC	783
Ser	His	Thr	Phe	Lys	Leu	Val	Asn	Ala	Asp	Gly	Glu	Ala	Val	Tyr	Cys	
			220					225					230			
AAG	TTC	CAT	TAC	AAG	ACC	GAC	CAG	GGC	ATC	AAA	AAC	TTG	CCT	GTT	GGA	831
Lys	Phe	His	Tyr	Lys	Thr	Asp	Gln	Gly	Ile	Lys	Asn	Leu	Pro	Val	Gly	
		235					240					245				
GAG	GCA	GGA	AGG	CTT	GCT	CAG	GAA	GAT	CCG	GAT	TAT	GGC	CTC	CGA	GAT	879
Glu	Ala	Gly	Arg	Leu	Ala	Gln	Glu	Asp	Pro	Asp	Tyr	Gly	Leu	Arg	Asp	
	250					255					260					
CTT	TTC	AAT	GCC	ATC	GCC	AAT	GGC	AAT	TAC	CCG	TCC	TGG	ACG	TTT	TAC	927
Leu	Phe	Asn	Ala	Ile	Ala	Asn	Gly	Asn	Tyr	Pro	Ser	Trp	Thr	Phe	Tyr	
265					270					275					280	
ATC	CAG	GTC	ATG	ACT	TTT	AAG	GAG	GCA	GAA	ACT	TTC	CCA	TTT	AAT	CCA	975
Ile	Gln	Val	Met	Thr	Phe	Lys	Glu	Ala	Glu	Thr	Phe	Pro	Phe	Asn	Pro	
				285					290					295		
TTT	GAT	CTG	ACC	AAG	GTT	TGG	CCT	CAC	AAG	GAC	TAC	CCT	CTT	ATA	CCA	1023
Phe	Asp	Leu	Thr	Lys	Val	Trp	Pro	His	Lys	Asp	Tyr	Pro	Leu	Ile	Pro	
			300					305					310			
GTT	GGC	AAA	GTG	GTT	TTA	AAC	AAA	AAT	CCA	GTT	AAT	TAC	TTT	GCT	GAA	1071
Val	Gly	Lys	Val	Val	Leu	Asn	Lys	Asn	Pro	Val	Asn	Tyr	Phe	Ala	Glu	
		315					320					325				
GTT	GAA	CAG	ATG	GCT	TTT	GAC	CCA	AGC	AAT	ATG	CCC	CCT	GGC	ATC	GAG	1119
Val	Glu	Gln	Met	Ala	Phe	Asp	Pro	Ser	Asn	Met	Pro	Pro	Gly	Ile	Glu	
	330					335					340					
CCC	AGC	CCT	GAC	AAA	AAG	CTT	CAG	GGC	CGC	CTT	TTT	GCC	TAC	CCG	GAC	1167
Pro	Ser	Pro	Asp	Lys	Lys	Leu	Gln	Gly	Arg	Leu	Phe	Ala	Tyr	Pro	Asp	
345					350					355					360	
ACT	CAC	CGC	CAC	CGC	CTG	GGA	CCC	AAC	TAT	CTG	CAG	ATA	CCT	GTG	AAC	1215
Thr	His	Arg	His	Arg	Leu	Gly	Pro	Asn	Tyr	Leu	Gln	Ile	Pro	Val	Asn	
				365					370					375		
TGT	CCC	TAC	CGC	GCT	CGA	GTG	GCC	AAC	TAC	CAG	CGT	GAT	GGC	CCC	ATG	1263
Cys	Pro	Tyr	Arg	Ala	Arg	Val	Ala	Asn	Tyr	Gln	Arg	Asp	Gly	Pro	Met	
			380					385					390			
TGC	ATG	CAT	GAC	AAC	CAG	GGT	GGT	GCC	CCC	AAC	TAT	TAC	CCC	AAC	AGC	1311
Cys	Met	His	Asp	Asn	Gln	Gly	Gly	Ala	Pro	Asn	Tyr	Tyr	Pro	Asn	Ser	
		395				400						405				

TTC AGC GCA CCA GAG CAG CAG CGC TCA GCC CTG GAG CAC AGC GTC CAG	1359
Phe Ser Ala Pro Glu Gln Gln Arg Ser Ala Leu Glu His Ser Val Gln	
410 415 420	
TGC GCT GTA GAT GTG AAA CGC TTC AAC AGT GCT AAT GAA GAC AAT GTC	1407
Cys Ala Val Asp Val Lys Arg Phe Asn Ser Ala Asn Glu Asp Asn Val	
425 430 435 440	
ACT CAG GTG CGG ACA TTC TAC ACA AAG GTG TTG AAT GAG GAG GAG AGG	1455
Thr Gln Val Arg Thr Phe Tyr Thr Lys Val Leu Asn Glu Glu Glu Arg	
445 450 455	
AAA CGC CTG TGT GAG AAC ATT GCC GGC CAC CTG AAG GAC GCT CAG CTT	1503
Lys Arg Leu Cys Glu Asn Ile Ala Gly His Leu Lys Asp Ala Gln Leu	
460 465 470	
TTC ATT CAG AAG AAA GCG GTC AAG AAT TTC ACT GAC GTC CAC CCT GAC	1551
Phe Ile Gln Lys Lys Ala Val Lys Asn Phe Thr Asp Val His Pro Asp	
475 480 485	
TAT GGG GCC CGC ATC CAG GCT CTT CTG GAC AAG TAC AAC GCT GAG AAG	1599
Tyr Gly Ala Arg Ile Gln Ala Leu Leu Asp Lys Tyr Asn Ala Glu Lys	
490 495 500	
CCT AAG AAC GCA ATT CAC ACC TAC ACG CAG GCC GGC TCT CAC ATG GCT	1647
Pro Lys Asn Ala Ile His Thr Tyr Thr Gln Ala Gly Ser His Met Ala	
505 510 515 520	
GCG AAG GGA AAA GCT AAC CTG TAACTCCGGT GCTCAGCCTC CGCTGAGGAG	1698
Ala Lys Gly Lys Ala Asn Leu	
525	
ACCTCTCGTG AAGCCGAGCC TGAGGATCAC CTGTAATCAA CGCTGGATGG ATTCTCCCCC	1758
GCCGGAGCGC AGACTCACGC TGATGACTTT AAAACGATAA TCCGGGCTTC TAGAGTGAAT	1818
GATAACCATG CTTTTGATGC CGTTTCCTGA AGGGAAATGA AAGGTTAGGG CTTAGCAATC	1878
ATTTAACAGA AACATGGATC TAATAGGACT TCTGTTTGGG TTATTCATTT AAATGACTAC	1938
ATTTAAAATG ATTACAAGAA AGGTGTTCTA GCCAGAAACA TGA CTGATT AGACAAGATA	1998
AAAATCTTGG CGAGAATAGT GTATTCTCCT ATTACCTCAT GGTCTGGTAT ATATACAATA	2058
CAACACACAT ACCACACACA CACACACATG CAATACACAC ACTACACACA CATAACACACA	2118
CTCACACACA CTCATACACA CACATGAAGA GATGATAAAG ATGGCCCACT CAGAATTTTT	2178
TTTTTATTTT TCTAAGGTCC TTATAAGCAA AACCATACTT GCATCATGTC TTCCAAAAGT	2238
AACTTTAGCA CTGTTGAAAC TTAATGTTTA TTCCTGTGCT GTGCGGTGCT GTGCTGTGCT	2298
GTGCTGTGCA GCTAATCAGA TTCTTGTTTT TTCCCACTTG GATTATGTTG ATGCTAATAC	2358
GCAGTGATTT CACATAGGAT GATTTGTACT TGCTTACATT TTTACAATAA AATGATCTAC	2418
ATGGA	2423

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 527 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Met Ser Asp Ser Arg Asp Pro Ala Ser Asp Gln Met Lys Gln Trp Lys
 1 5 10 15
 Glu Gln Arg Ala Ser Gln Arg Pro Asp Val Leu Thr Thr Gly Gly Gly
 20 25 30
 Asn Pro Ile Gly Asp Lys Leu Asn Ile Met Thr Ala Gly Ser Arg Gly
 35 40 45
 Pro Leu Leu Val Gln Asp Val Val Phe Thr Asp Glu Met Ala His Phe
 50 55 60
 Asp Arg Glu Arg Ile Pro Glu Arg Val Val His Ala Lys Gly Ala Gly
 65 70 75 80
 Ala Phe Gly Tyr Phe Glu Val Thr His Asp Ile Thr Arg Tyr Ser Lys
 85 90 95
 Gly Lys Val Phe Glu His Ile Gly Lys Arg Thr Pro Ile Ala Val Arg
 100 105 110
 Phe Ser Thr Val Ala Gly Glu Ser Gly Ser Ala Asp Thr Val Arg Asp
 115 120 125
 Pro Arg Gly Phe Ala Val Lys Phe Tyr Thr Glu Asp Gly Asn Trp Asp
 130 135 140
 Leu Val Gly Asn Asn Thr Pro Ile Phe Phe Ile Arg Asp Ala Ile Leu
 145 150 155 160
 Phe Pro Ser Phe Ile His Ser Gln Lys Arg Asn Pro Gln Thr His Leu
 165 170 175
 Lys Asp Pro Asp Met Val Trp Asp Phe Trp Ser Leu Arg Pro Glu Ser
 180 185 190
 Leu His Gln Val Ser Phe Leu Phe Ser Asp Arg Gly Ile Pro Asp Gly
 195 200 205
 His Arg His Met Asn Gly Tyr Gly Ser His Thr Phe Lys Leu Val Asn
 210 215 220
 Ala Asp Gly Glu Ala Val Tyr Cys Lys Phe His Tyr Lys Thr Asp Gln
 225 230 235 240
 Gly Ile Lys Asn Leu Pro Val Gly Glu Ala Gly Arg Leu Ala Gln Glu
 245 250 255
 Asp Pro Asp Tyr Gly Leu Arg Asp Leu Phe Asn Ala Ile Ala Asn Gly
 260 265 270
 Asn Tyr Pro Ser Trp Thr Phe Tyr Ile Gln Val Met Thr Phe Lys Glu
 275 280 285
 Ala Glu Thr Phe Pro Phe Asn Pro Phe Asp Leu Thr Lys Val Trp Pro
 290 295 300
 His Lys Asp Tyr Pro Leu Ile Pro Val Gly Lys Val Val Leu Asn Lys
 305 310 315 320
 Asn Pro Val Asn Tyr Phe Ala Glu Val Glu Gln Met Ala Phe Asp Pro
 325 330 335
 Ser Asn Met Pro Pro Gly Ile Glu Pro Ser Pro Asp Lys Lys Leu Gln
 340 345 350

Gly Arg Leu Phe Ala Tyr Pro Asp Thr His Arg His Arg Leu Gly Pro
 355 360 365

Asn Tyr Leu Gln Ile Pro Val Asn Cys Pro Tyr Arg Ala Arg Val Ala
 370 375 380

Asn Tyr Gln Arg Asp Gly Pro Met Cys Met His Asp Asn Gln Gly Gly
 385 390 395 400

Ala Pro Asn Tyr Tyr Pro Asn Ser Phe Ser Ala Pro Glu Gln Gln Arg
 405 410 415

Ser Ala Leu Glu His Ser Val Gln Cys Ala Val Asp Val Lys Arg Phe
 420 425 430

Asn Ser Ala Asn Glu Asp Asn Val Thr Gln Val Arg Thr Phe Tyr Thr
 435 440 445

Lys Val Leu Asn Glu Glu Glu Arg Lys Arg Leu Cys Glu Asn Ile Ala
 450 455 460

Gly His Leu Lys Asp Ala Gln Leu Phe Ile Gln Lys Lys Ala Val Lys
 465 470 475 480

Asn Phe Thr Asp Val His Pro Asp Tyr Gly Ala Arg Ile Gln Ala Leu
 485 490 495

Leu Asp Lys Tyr Asn Ala Glu Lys Pro Lys Asn Ala Ile His Thr Tyr
 500 505 510

Thr Gln Ala Gly Ser His Met Ala Ala Lys Gly Lys Ala Asn Leu
 515 520 525

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 969 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: human manganese superoxide dismutase
 EMBL #X59445

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 61..729

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TGGCTTCGGC AGCGGCTTCA GCAGATCGGC GGCATCAGCG GTAGCACCAG CACTAGCAGC	60
ATG TTG AGC CGG GCA GTG TGC GGC ACC AGC AGG CAG CTG GCT CCG GCT	108
Met Leu Ser Arg Ala Val Cys Gly Thr Ser Arg Gln Leu Ala Pro Ala	
1 5 10 15	
TTG GGG TAT CTG GGC TCC AGG CAG AAG CAC AGC CTC CCC GAC CTG CCC	156
Leu Gly Tyr Leu Gly Ser Arg Gln Lys His Ser Leu Pro Asp Leu Pro	

95

20	25	30	
TAC GAC TAC GGC GCC CTG GAA CCT CAC ATC AAC GCG CAG ATC ATG CAG Tyr Asp Tyr Gly Ala Leu Glu Pro His Ile Asn Ala Gln Ile Met Gln 35 40 45			204
CTG CAC CAC AGC AAG CAC CAC GCG GCC TAC GTG AAC AAC CTG AAC GTC Leu His His Ser Lys His His Ala Ala Tyr Val Asn Asn Leu Asn Val 50 55 60			252
AAC GAG GAG AAG TAC CAG GAG GCG TTG GCC AAG GGA GAT GTT ACA GCC Asn Glu Glu Lys Tyr Gln Glu Ala Leu Ala Lys Gly Asp Val Thr Ala 65 70 75 80			300
CAG ATA GCT CTT CAG CCT GCA CTG AAG TTC AAT GGT GGT GGT CAT ATC Gln Ile Ala Leu Gln Pro Ala Leu Lys Phe Asn Gly Gly Gly His Ile 85 90 95			348
AAT CAT AGC ATT TTC TGG ACA AAC CTC AGC CCT AAC GGT GGT GGA GAA Asn His Ser Ile Phe Trp Thr Asn Leu Ser Pro Asn Gly Gly Gly Glu 100 105 110			396
CCC AAA GGG GAG TTG CTG GAA GCC ATC AAA CGT GAC TTT GGT TCC TTT Pro Lys Gly Glu Leu Leu Glu Ala Ile Lys Arg Asp Phe Gly Ser Phe 115 120 125			444
GAC AAG TTT AAG GAG AAG CTG ACG GCT GCA TCT GTT GGT GTC CAA GGC Asp Lys Phe Lys Glu Lys Leu Thr Ala Ala Ser Val Gly Val Gln Gly 130 135 140			492
TCA GGT TGG GGT TGG CTT GGT TTC AAT AAG GAA CGG GGA CAC TTA CAA Ser Gly Trp Gly Trp Leu Gly Phe Asn Lys Glu Arg Gly His Leu Gln 145 150 155 160			540
ATT GCT GCT TGT CCA AAT CAG GAT CCA CTG CAA GGA ACA ACA GGC CTT Ile Ala Ala Cys Pro Asn Gln Asp Pro Leu Gln Gly Thr Thr Gly Leu 165 170 175			588
ATT CCA CTG CTG GGG ATT GAT GTG TGG GAG CAC GCT TAC TAC CTT CAG Ile Pro Leu Leu Gly Ile Asp Val Trp Glu His Ala Tyr Tyr Leu Gln 180 185 190			636
TAT AAA AAT GTC AGG CCT GAT TAT CTA AAA GCT ATT TGG AAT GTA ATC Tyr Lys Asn Val Arg Pro Asp Tyr Leu Lys Ala Ile Trp Asn Val Ile 195 200 205			684
AAC TGG GAG AAT GTA ACT GAA AGA TAC ATG GCT TGC AAA AAG TAAACCACGA Asn Trp Glu Asn Val Thr Glu Arg Tyr Met Ala Cys Lys Lys 210 215 220			736
TCGTTATGCT GAGTATGTTA AGCTCTTTAT GACTGTTTTT GTAGTGGTAT AGAGTACTGC			796
AGAATACAGT AAGCTGCTCT ATTGTAGCAT TTCTTGATGT TGCTTAGTCA CTTATTTTCAT			856
AAACAACCTTA ATGTTCTGAA TAATTTCTTA CTAAACATTT TGTTATTGGG CAAGTGATTG			916
AAAATAGTAA ATGCTTTGTG TGATTGAAAA AAAAAAAAAA AAAAAAAAAA AAA			969

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 222 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Met Leu Ser Arg Ala Val Cys Gly Thr Ser Arg Gln Leu Ala Pro Ala
 1 5 10 15
 Leu Gly Tyr Leu Gly Ser Arg Gln Lys His Ser Leu Pro Asp Leu Pro
 20 25 30
 Tyr Asp Tyr Gly Ala Leu Glu Pro His Ile Asn Ala Gln Ile Met Gln
 35 40 45
 Leu His His Ser Lys His His Ala Ala Tyr Val Asn Asn Leu Asn Val
 50 55 60
 Asn Glu Glu Lys Tyr Gln Glu Ala Leu Ala Lys Gly Asp Val Thr Ala
 65 70 75 80
 Gln Ile Ala Leu Gln Pro Ala Leu Lys Phe Asn Gly Gly Gly His Ile
 85 90 95
 Asn His Ser Ile Phe Trp Thr Asn Leu Ser Pro Asn Gly Gly Gly Glu
 100 105 110
 Pro Lys Gly Glu Leu Leu Glu Ala Ile Lys Arg Asp Phe Gly Ser Phe
 115 120 125
 Asp Lys Phe Lys Glu Lys Leu Thr Ala Ala Ser Val Gly Val Gln Gly
 130 135 140
 Ser Gly Trp Gly Trp Leu Gly Phe Asn Lys Glu Arg Gly His Leu Gln
 145 150 155 160
 Ile Ala Ala Cys Pro Asn Gln Asp Pro Leu Gln Gly Thr Thr Gly Leu
 165 170 175
 Ile Pro Leu Leu Gly Ile Asp Val Trp Glu His Ala Tyr Tyr Leu Gln
 180 185 190
 Tyr Lys Asn Val Arg Pro Asp Tyr Leu Lys Ala Ile Trp Asn Val Ile
 195 200 205
 Asn Trp Glu Asn Val Thr Glu Arg Tyr Met Ala Cys Lys Lys
 210 215 220

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 691 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: human enolase gene (EMBL #X56832)
 fragment containinig nucleotides -628 to +63

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 629..691

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```

CTCTGGGGGTG GAGGTAGTAA AGGGTGAGCA TGGTATTGGC TTGGAGGAAG TGGGGGACAT      60
TTCTGCTTTT TTTCTCTCTG GGAAGTGGAGA TGCTTGAAAA AGCTGGGGGA AGGGGCGGCT      120
GGAGCAAGCA GATGGGACAA ACTCTGGGAA CACCGAAGGA TCTAGGGAAA GGAGGCTGTG      180
AGGAGGGCAG CAGGGATGGA TAGAAAAGGG CAGCTAGAGC TGGAACCTGA TAGGGAATTG      240
GGGGCCCAAG GAGATTTTCG AGCAGGAAAA TGAGAACCAG AAAGGATTG AAGGCCACCA      300
GCCATGGAGA ACAGACTGCT TGACCAGAGG GGTGGAAGGA GAAGGCCTAA GTGGAGGCTT      360
GGGGGAGGTG GGGGCTTGGT GAGCGGTGGC ATCCCAGGAG CTATAGATAA GAGGCCCTG      420
GATTCTTAGG ATGGGAGGGT GGAATAAGAG CTGTTCTGAG TGGGGGAGGG GGCTGCGCCT      480
GCCTCTTTGG TCTGTGACCT TTTTGTAGGG TATTTTTCAG TCCAGCACCT GCCTTCTTGG      540
AGTGGGGAAG AATCTTAAAG GGCAAGGGAT TTCTGGTTCC TTAAGAGATC AACTGTCTAC      600
ACTCACTCAC ACCTCTGTGC CTGCAGCC ATG GCC ATG CAG AAA ATC TTT GCC      652
                        Met Ala Met Gln Lys Ile Phe Ala
                        1                      5

CGG GAA ATC TTG GAC TCC AGG GGC AAC CCC ACG GTG GAG      691
Arg Glu Ile Leu Asp Ser Arg Gly Asn Pro Thr Val Glu
  10                      15                      20

```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```

Met Ala Met Gln Lys Ile Phe Ala Arg Glu Ile Leu Asp Ser Arg Gly
  1                      5                      10                      15
Asn Pro Thr Val Glu
                20

```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: PKM/ENO3 consensus sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GAGAGGCCGGG CTNNCTG

17

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 786 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: -760 MTAIIa promoter fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AAGCTTGTGG CTTCTTCTCC TTACTCTTCC TCCTTGGTGT CTCTATGTTA GAGGGCCGTT	60
AGCATCTGCT GGGGCCTGGT CGCATTACACC CTGCTCTGCC ACTCACTGGC TGTGTGACTC	120
TGGACAAATT AACTTCTCTG GACCTGGCAG TTTCTCCTCT CTACAATGAG AATACTGGAG	180
AGTCCTTATC TTATGGGTTG CTACAGAATT AAGTGACATC TCACACACAA CACACTTCCT	240
ACAGTCCCTG TTACACGCTA AAAGTACTCA ACTAGCTTCG GATACGTCAT CAGCAACCAC	300
CCCACGGGTT ACTGTGATGC TGCACAATTA TTAAGCCCTG GCTGCTACAG AGTTGTAACC	360
TGTCTGCACT TCCAACCGGC GCCGCAAGCA GCATTCCCAG TCCCGCTTTC ACCCGCGCGC	420
TAACGGCTCA GGTTCGAGTA CAGGACAGGA GGGAGGGGAG CTGTGCACAC GGCGGAGGCG	480
CACGGCGTGG GCACCCAGCA CCCGGTACAC TGTGTCCTCC CGCTGCACCC AGCCCCTTCA	540
GCCCCGAGGCG TCCCCGAGGC GCAAGTGGGC CGCCTTCAGG GAACTGACCG CCCGCGGCCC	600
GTGTGCAGAG CCGGGTGCGC CCGGCCAGT GCGCGCGGCC GGGTGTTTCG CTTGGAGCCG	660
CAAGTGA CTGACGCGGG GCGTGTGCAG GCACGGCCGG GCGGGGGCTT TTGCACTCGT	720
CCCGGCTCTT TCTAGCTATA AACACTGCTT GCCGCGCTGC ACTCCACCAC GCCTCCTCCA	780
AGTCCC	786

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 366 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: -345 MTAIIa promoter fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TAACGGCTCA GGTTCGAGTA CAGGACAGGA GGGAGGGGAG CTGTGCACAC GCGGAGGCG	60
CACGGCGTGG GCACCCAGCA CCCGGTACAC TGTGTCCTCC CGCTGCACCC AGCCCCTTCA	120
GCCCGAGGCG TCCCCGAGGC GCAAGTGGGC CGCCTTCAGG GAACTGACCG CCCGCGGCCC	180
GTGTGCAGAG CCGGGTGC GC CCGGCCCCAGT GCGCGCGGCC GGGTGTTCG CTTGGAGCCG	240
CAAGTGA CTT CTAGCGCGGG GCGTGTGCAG GCACGGCCGG GCGGGGCTT TTGCACTCGT	300
CCCGGCTCTT TCTAGCTATA AACACTGCTT GCCGCGCTGC ACTCCACCAC GCCTCCTCCA	360
AGTCCC	366

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 184 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: -163 MTAIIa promoter fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GTGCAGAGCC GGGTGC GCCC GGGCCAGTGC GCGCGGCCGG GTGTTTCGCT TGGAGCCGCA	60
AGTGACTTCT AGCGCGGGGC GTGTGCAGGC ACGGCCGGGG CGGGGCTTTT GCACTCGTCC	120
CGGCTCTTTC TAGCTATAAA CACTGCTTGC CGCGCTGCAC TCCACCACGC CTCCTCCAAG	180
TCCC	184

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 111 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: -90 MTAIIa promoter fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GCGGGGCGTG TGCAGGCACG GCCGGGGCGG GGCTTTTGCA CTCGTCCCGG CTCTTTCTAG	60
CTATAAACAC TGCTTGCCGC GCTGCACTCC ACCACGCCTC CTCCAAGTCC C	111

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1643 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: TNF cDNA HSTNFR (EMBL Accession #X01394)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 153..851

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GCAGAGGACC AGCTAAGAGG GAGAGAAGCA ACTACAGACC CCCCTGAAA ACAACCCTCA	60
GACGCCACAT CCCCTGACAA GCTGCCAGGC AGGTTCTCTT CCTCTCACAT ACTGACCCAC	120
GGCTCCACCC TCTCTCCCCT GGAAAGGACA CC ATG AGC ACT GAA AGC ATG ATC	173
Met Ser Thr Glu Ser Met Ile	
1 5	
CGG GAC GTG GAG CTG GCC GAG GAG GCG CTC CCC AAG AAG ACA GGG GGG	221
Arg Asp Val Glu Leu Ala Glu Glu Ala Leu Pro Lys Lys Thr Gly Gly	
10 15 20	
CCC CAG GGC TCC AGG CGG TGC TTG TTC CTC AGC CTC TTC TCC TTC CTG	269
Pro Gln Gly Ser Arg Arg Cys Leu Phe Leu Ser Leu Phe Ser Phe Leu	
25 30 35	
ATC GTG GCA GGC GCC ACC ACG CTC TTC TGC CTG CTG CAC TTT GGA GTG	317
Ile Val Ala Gly Ala Thr Thr Leu Phe Cys Leu Leu His Phe Gly Val	
40 45 50 55	
ATC GGC CCC CAG AGG GAA GAG TTC CCC AGG GAC CTC TCT CTA ATC AGC	365
Ile Gly Pro Gln Arg Glu Glu Phe Pro Arg Asp Leu Ser Leu Ile Ser	
60 65 70	
CCT CTG GCC CAG GCA GTC AGA TCA TCT TCT CGA ACC CCG AGT GAC AAG	413
Pro Leu Ala Gln Ala Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Lys	
75 80 85	
CCT GTA GCC CAT GTT GTA GCA AAC CCT CAA GCT GAG GGG CAG CTC CAG	461
Pro Val Ala His Val Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln	
90 95 100	
TGG CTG AAC CGC CGG GCC AAT GCC CTC CTG GCC AAT GGC GTG GAG CTG	509
Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu	
105 110 115	
AGA GAT AAC CAG CTG GTG GTG CCA TCA GAG GGC CTG TAC CTC ATC TAC	557
Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr	
120 125 130 135	
TCC CAG GTC CTC TTC AAG GGC CAA GGC TGC CCC TCC ACC CAT GTG CTC	605
Ser Gln Val Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu	

101																		
140									145				150					
CTC	ACC	CAC	ACC	ATC	AGC	CGC	ATC	GCC	GTC	TCC	TAC	CAG	ACC	AAG	GTC	653		
Leu	Thr	His	Thr	Ile	Ser	Arg	Ile	Ala	Val	Ser	Tyr	Gln	Thr	Lys	Val			
155				160				165										
AAC	CTC	CTC	TCT	GCC	ATC	AAG	AGC	CCC	TGC	CAG	AGG	GAG	ACC	CCA	GAG	701		
Asn	Leu	Leu	Ser	Ala	Ile	Lys	Ser	Pro	Cys	Gln	Arg	Glu	Thr	Pro	Glu			
170				175				180										
GGG	GCT	GAG	GCC	AAG	CCC	TGG	TAT	GAG	CCC	ATC	TAT	CTG	GGA	GGG	GTC	749		
Gly	Ala	Glu	Ala	Lys	Pro	Trp	Tyr	Glu	Pro	Ile	Tyr	Leu	Gly	Gly	Val			
185				190				195										
TTC	CAG	CTG	GAG	AAG	GGT	GAC	CGA	CTC	AGC	GCT	GAG	ATC	AAT	CGG	CCC	797		
Phe	Gln	Leu	Glu	Lys	Gly	Asp	Arg	Leu	Ser	Ala	Glu	Ile	Asn	Arg	Pro			
200				205				210				215						
GAC	TAT	CTC	GAC	TTT	GCC	GAG	TCT	GGG	CAG	GTC	TAC	TTT	GGG	ATC	ATT	845		
Asp	Tyr	Leu	Asp	Phe	Ala	Glu	Ser	Gly	Gln	Val	Tyr	Phe	Gly	Ile	Ile			
220				225				230										
GCC CTG TGAGGAGGAC GAACATCCAA CCTTCCCAA CGCCTCCCCT GCCCAATCC																		901
Ala Leu																		
CTTTATTACC CCCTCCTTCA GACACCCTCA ACCTCTTCTG GCTCAAAAAG AGAATTGGGG																		961
GCTTAGGGTC GGAACCCAAG CTTAGAACTT TAAGCAACAA GACCACCACT TCGAAACCTG																		1021
GGATTCAGGA ATGTGTGGCC TGCACAGTGA ATTGCTGGCA ACCACTAAGA ATTCAAACTG																		1081
GGGCCTCCAG AACTCACTGG GGCCTACAGC TTTGATCCCT GACATCTGGA ATCTGGAGAC																		1141
CAGGGAGCCT TTGGTTCTGG CCAGAATGCT GCAGGACTTG AGAAGACCTC ACCTAGAAAT																		1201
TGACACAAGT GGACCTTAGG CCTTCCTCTC TCCAGATGTT TCCAGACTTC CTTGAGACAC																		1261
GGAGCCCAGC CCTCCCCATG GAGCCAGCTC CCTCTATTTA TGTTTGCACT TGTGATTATT																		1321
TATTATTTAT TTATTATTTA TTTATTTACA GATGAATGTA TTTATTTGGG AGACCGGGGT																		1381
ATCCTGGGGG ACCCAATGTA GGAGCTGCCT TGGCTCAGAC ATGTTTTCCG TGAAAACGGA																		1441
GCTGAACAAT AGGCTGTTCC CATGTAGCCC CCTGGCCTCT GTGCCTTCTT TTGATTATGT																		1501
TTTTTAAAT ATTTATCTGA TTAAGTTGTC TAAACAATGC TGATTTGGTG ACCAACTGTC																		1561
ACTCATTGCT GAGCCTCTGC TCCCCAGGGG AGTTGTGTCT GTAATCGCCC TACTATTGAG																		1621
TGGCGAGAAA TAAAGTTTGC TT																		1643

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 233 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
 1 5 10 15

102

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
 20 25 30
 Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
 35 40 45
 Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
 50 55 60
 Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Val Arg Ser Ser
 65 70 75 80
 Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro
 85 90 95
 Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu
 100 105 110
 Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser
 115 120 125
 Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly
 130 135 140
 Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala
 145 150 155 160
 Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro
 165 170 175
 Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu
 180 185 190
 Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu
 195 200 205
 Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly
 210 215 220
 Gln Val Tyr Phe Gly Ile Ile Ala Leu
 225 230

IT IS CLAIMED:

1. A chimeric gene, comprising
a hypoxia response enhancer element, a tissue-specific promoter heterologous to the
5 element, and a therapeutic gene,
wherein said promoter is operably linked to said therapeutic gene and said element
is effective to modulate expression of said therapeutic gene.
2. A chimeric gene of claim 1, wherein said promoter is a cardiac-specific
10 promoter.
3. A chimeric gene of claim 2, wherein said promoter is selected from the group
consisting of α -MHC_{3.5} promoter, α -MHC₈₇ promoter, and human cardiac actin promoter.
- 15 4. A chimeric gene of claim 1, wherein said promoter is a kidney-specific
promoter.
5. A chimeric gene of claim 4, wherein said promoter is a renin promoter.
- 20 6. A chimeric gene of claim 1, wherein said promoter is a brain-specific promoter.
7. A chimeric gene of claim 6, wherein said promoter is selected from the group
consisting of aldolase C promoter, and tyrosine hydroxylase promoter.
- 25 8. A chimeric gene of claim 1, wherein said promoter is a vascular endothelium-
specific promoter.
9. A chimeric gene of claim 8, wherein said promoter is selected from the group
consisting of Et-1 promoter and vonWillebrand factor promoter.
- 30 10. A chimeric gene of claim 1, wherein said hypoxia response enhancer element is
selected from the group consisting of erythropoietin HRE element (HREE1), pyruvate
kinase (PKM) HRE element, enolase 3 (ENO3) HRE element, endothelin-1 (ET-1) HRE
element and metallothionein II (MTII) HRE element.

11. A chimeric gene of claim 10, wherein said HRE element has a sequence contained in SEQ ID NO:35.

12. A chimeric gene of claim 1, wherein said therapeutic gene is selected from the
5 group consisting of nitric oxide synthase (NOS), Bcl-2, superoxide dismutase (SOD), and catalase.

13. An expression vector, comprising the chimeric gene of any of claims 1-12.

10 14. An expression vector of claim 13, wherein said expression vector is a plasmid.

15. An expression vector of claim 13, wherein said expression vector is an adenovirus vector.

15 16. An expression vector of claim 13, wherein said expression vector is a retro-virus vector.

17. A method of reducing ischemic injury to a cell exposed to hypoxic conditions, comprising
20 introducing into said cell a chimeric gene of any of claims 1-12,
wherein exposing the cell to hypoxic conditions increases expression of said therapeutic gene and wherein expression of said therapeutic gene is effective to reduce ischemic injury to the cell.

25 18. A method of claim 17, wherein said cell is a vascular endothelium cell and said promoter is a vascular endothelium-specific promoter.

19. A method of reducing ischemic injury to a cell exposed to hypoxic conditions, comprising
30 introducing into said cell a chimeric gene containing a hypoxia response enhancer element, a therapeutic gene, and a tissue-specific promoter operably linked to said therapeutic gene, where said element is effective to modulate expression of said therapeutic gene,

wherein exposing the cell to hypoxic conditions increases expression of said therapeutic gene and wherein expression of said therapeutic gene is effective to reduce ischemic injury to the cell.

- 5 20. A hypoxia response enhancer (HRE) element consisting of a sequence derived from SEQ ID NO:35.

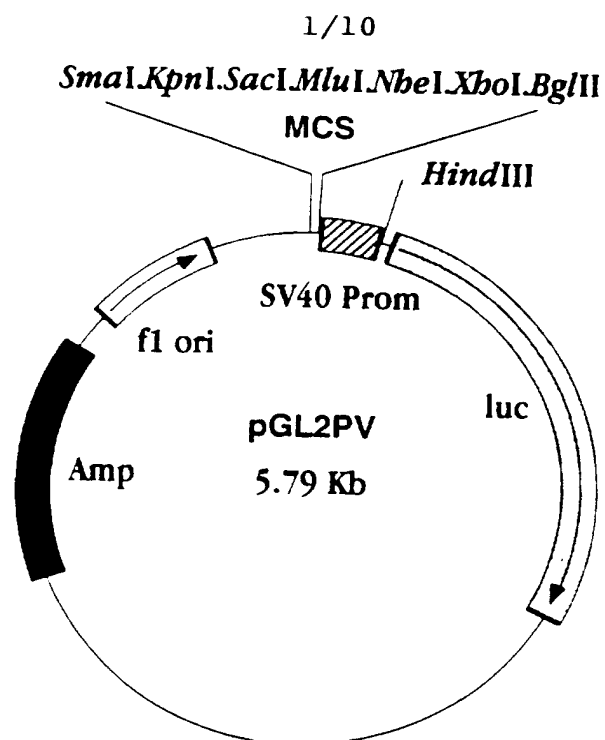


FIG. 1A

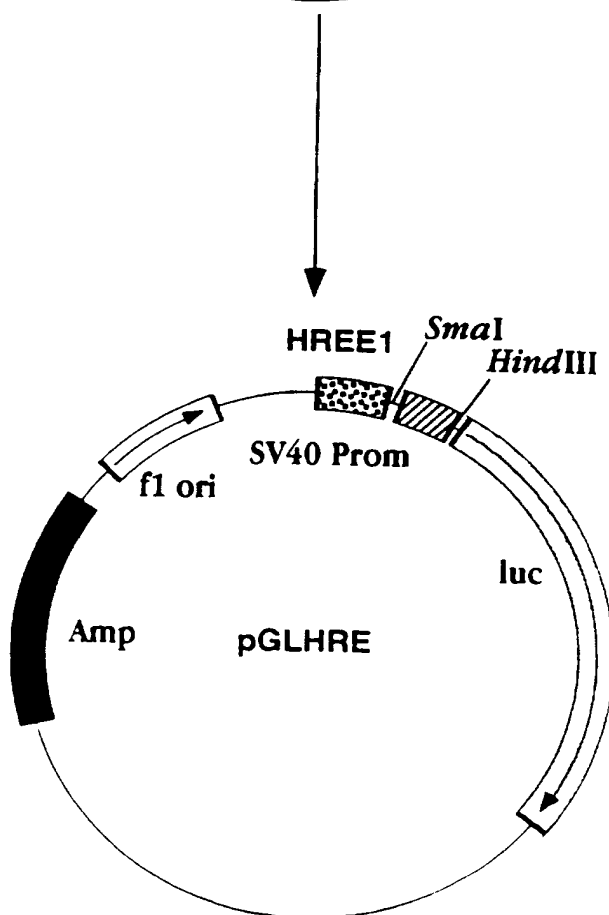
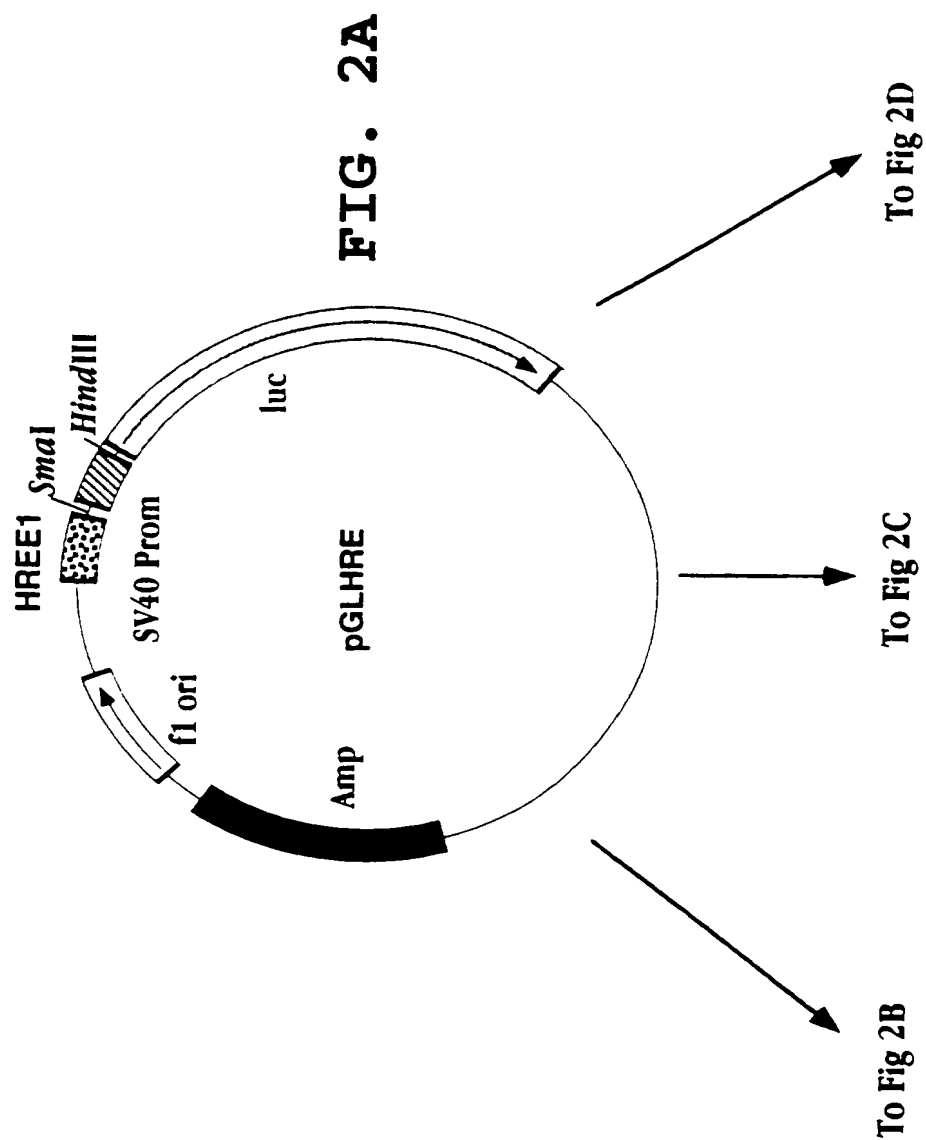


FIG. 1B

2/10



3/10

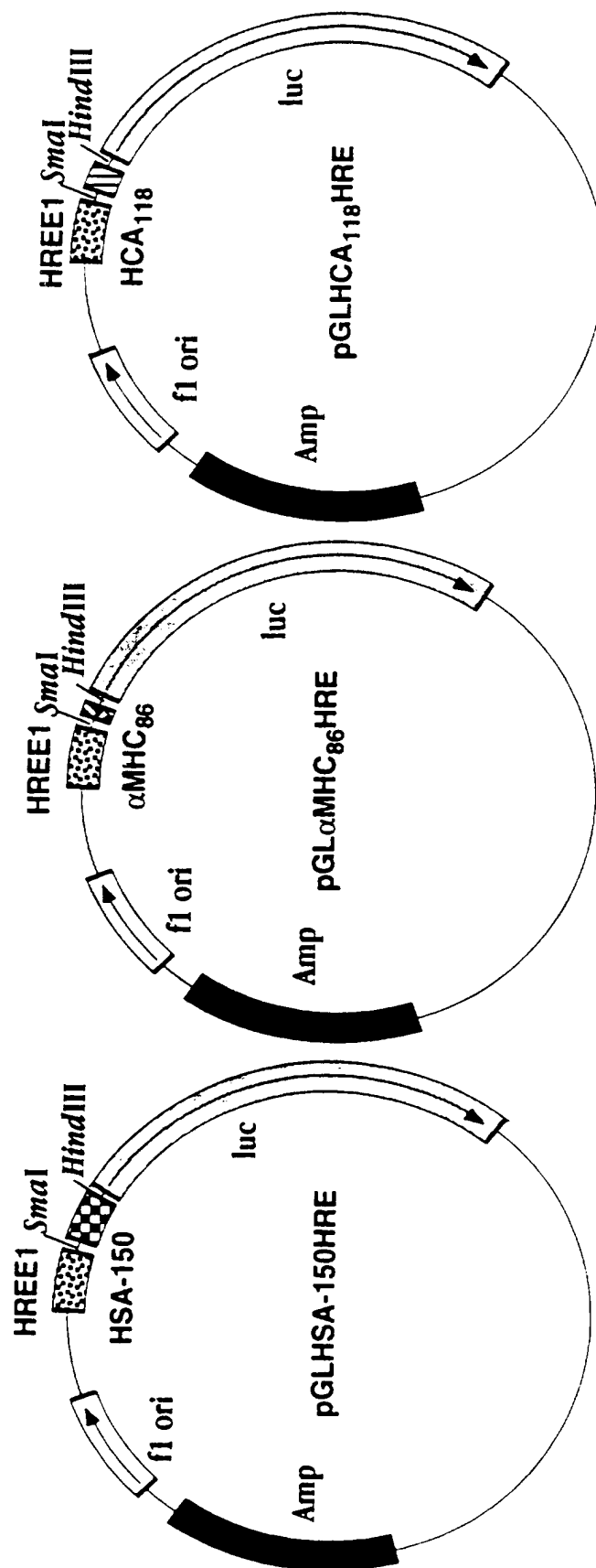


FIG. 2B

FIG. 2C

FIG. 2D

4/10

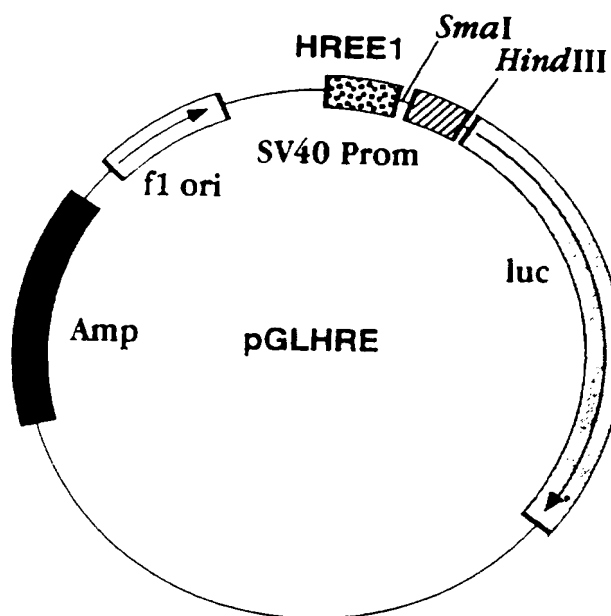


FIG. 3A

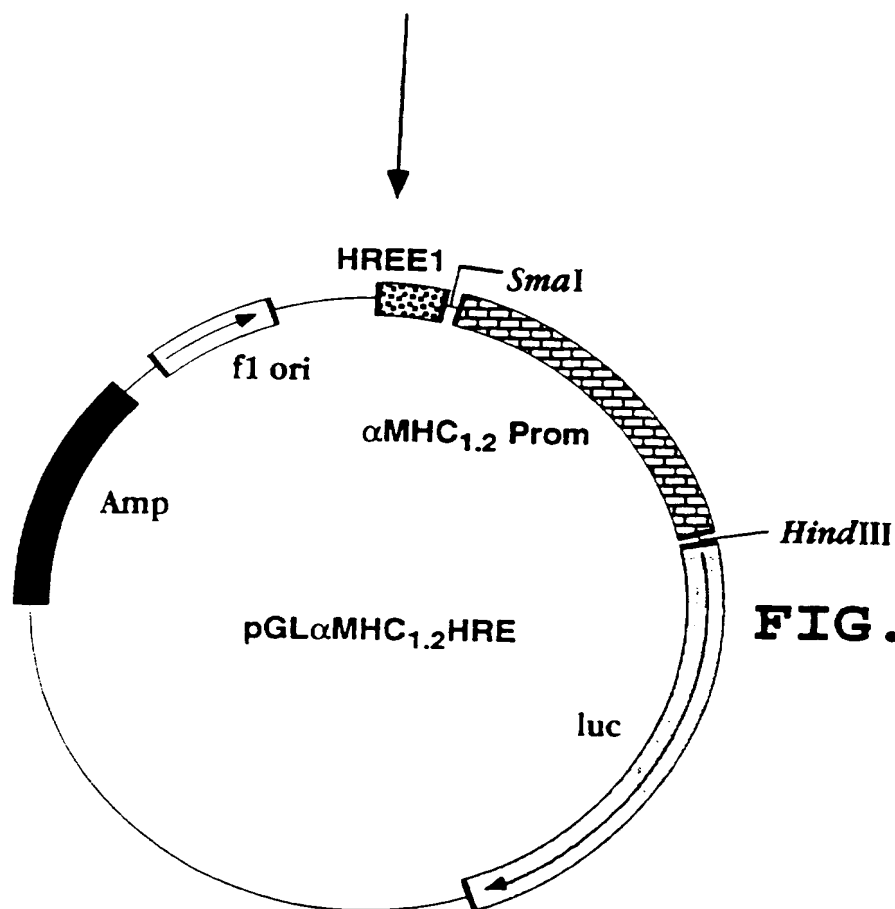


FIG. 3B

5/10

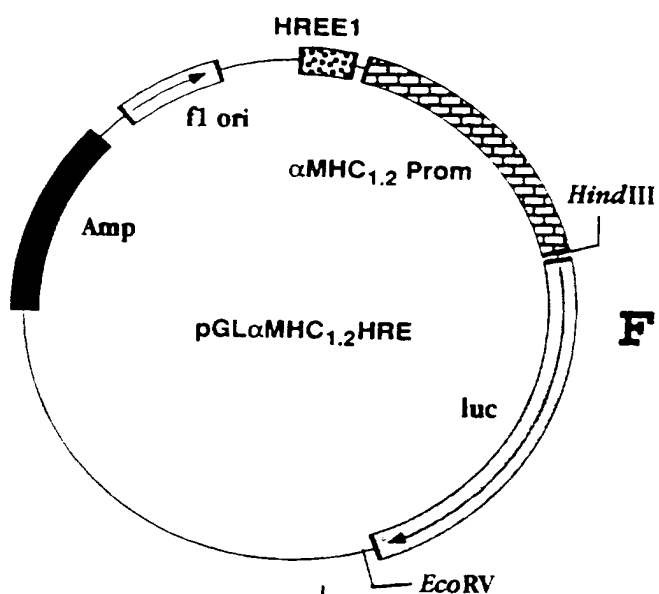


FIG. 4A

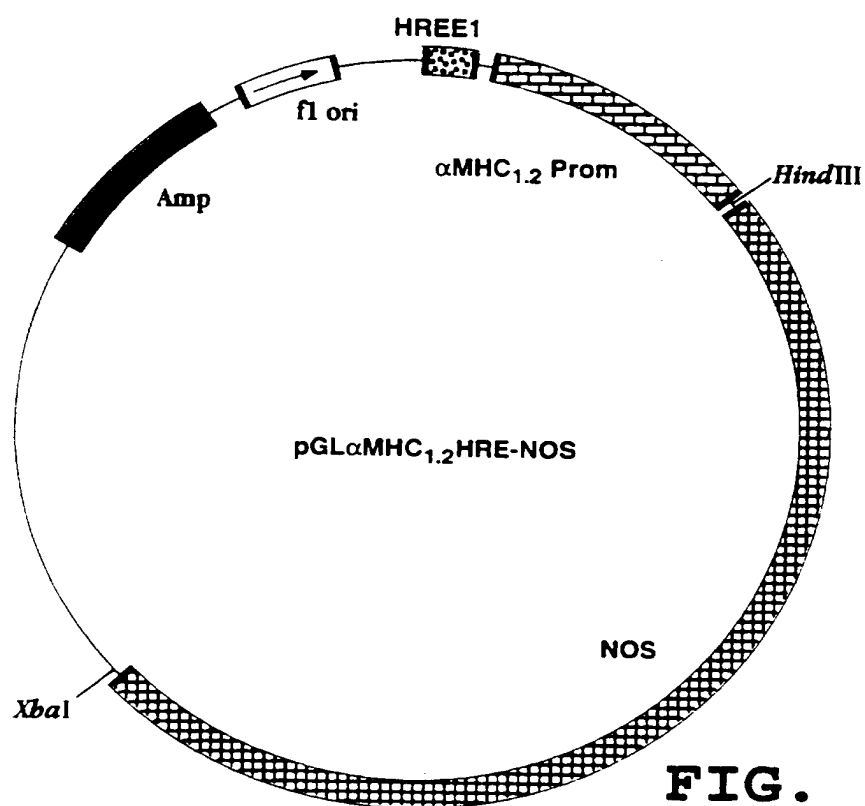
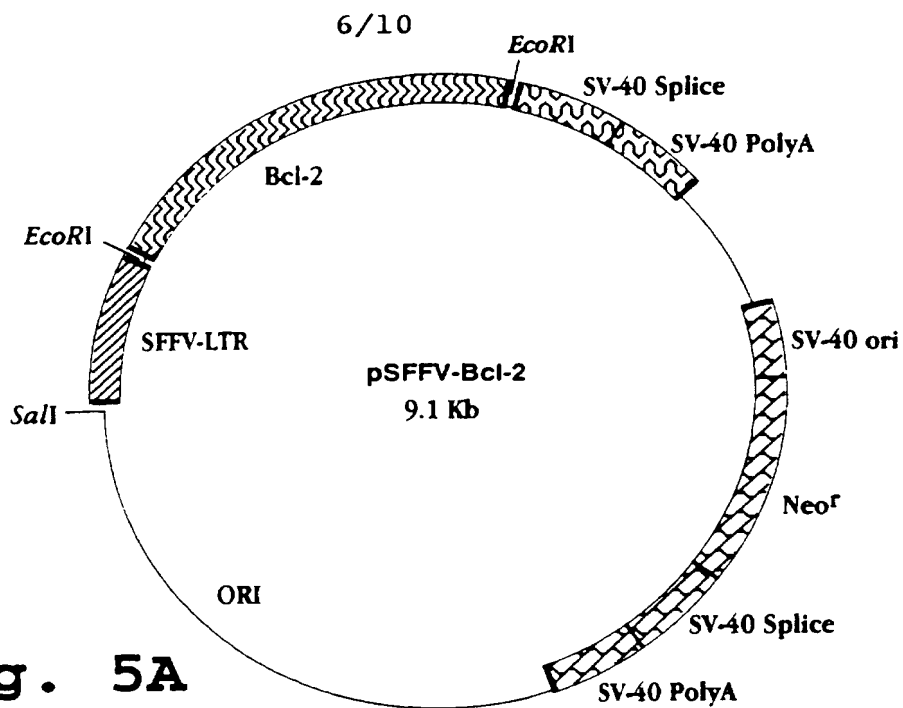
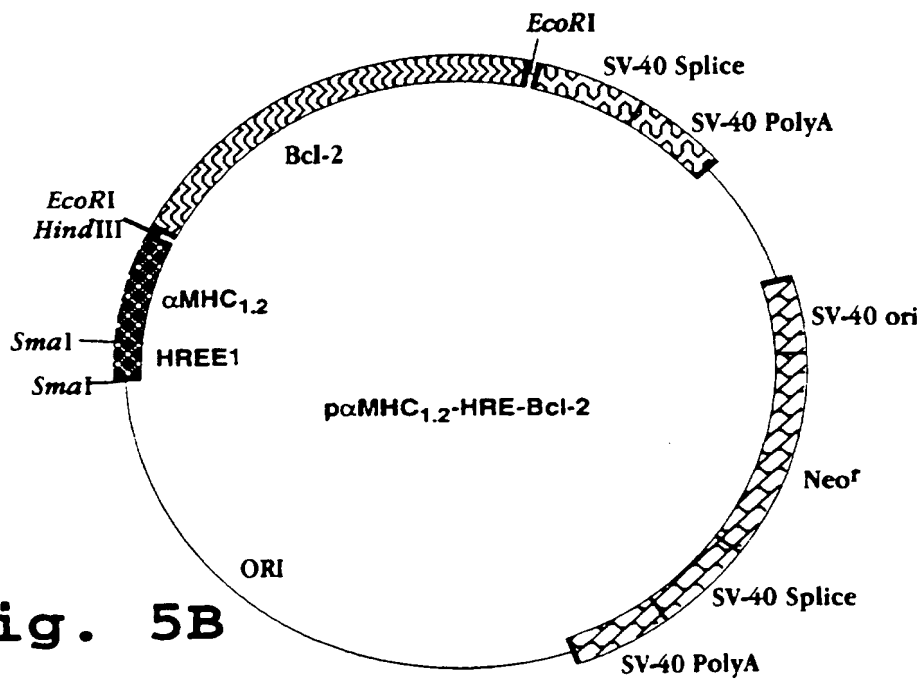


FIG. 4B

**Fig. 5A****Fig. 5B**

7/10

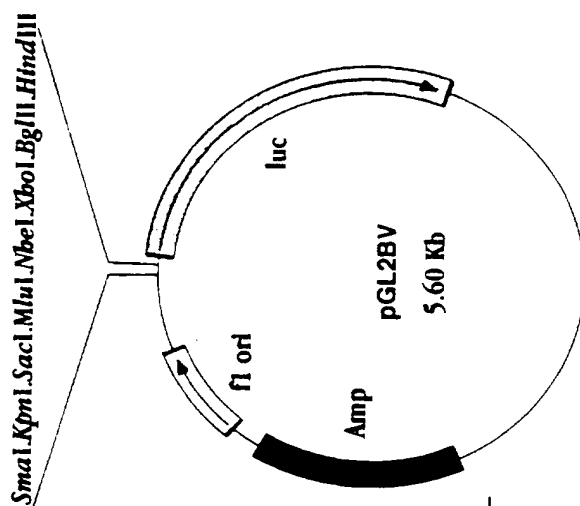
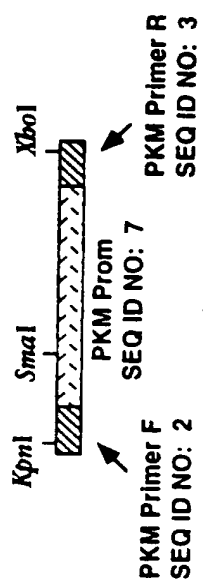


Fig. 6B

Fig. 6A



To Fig. 6C

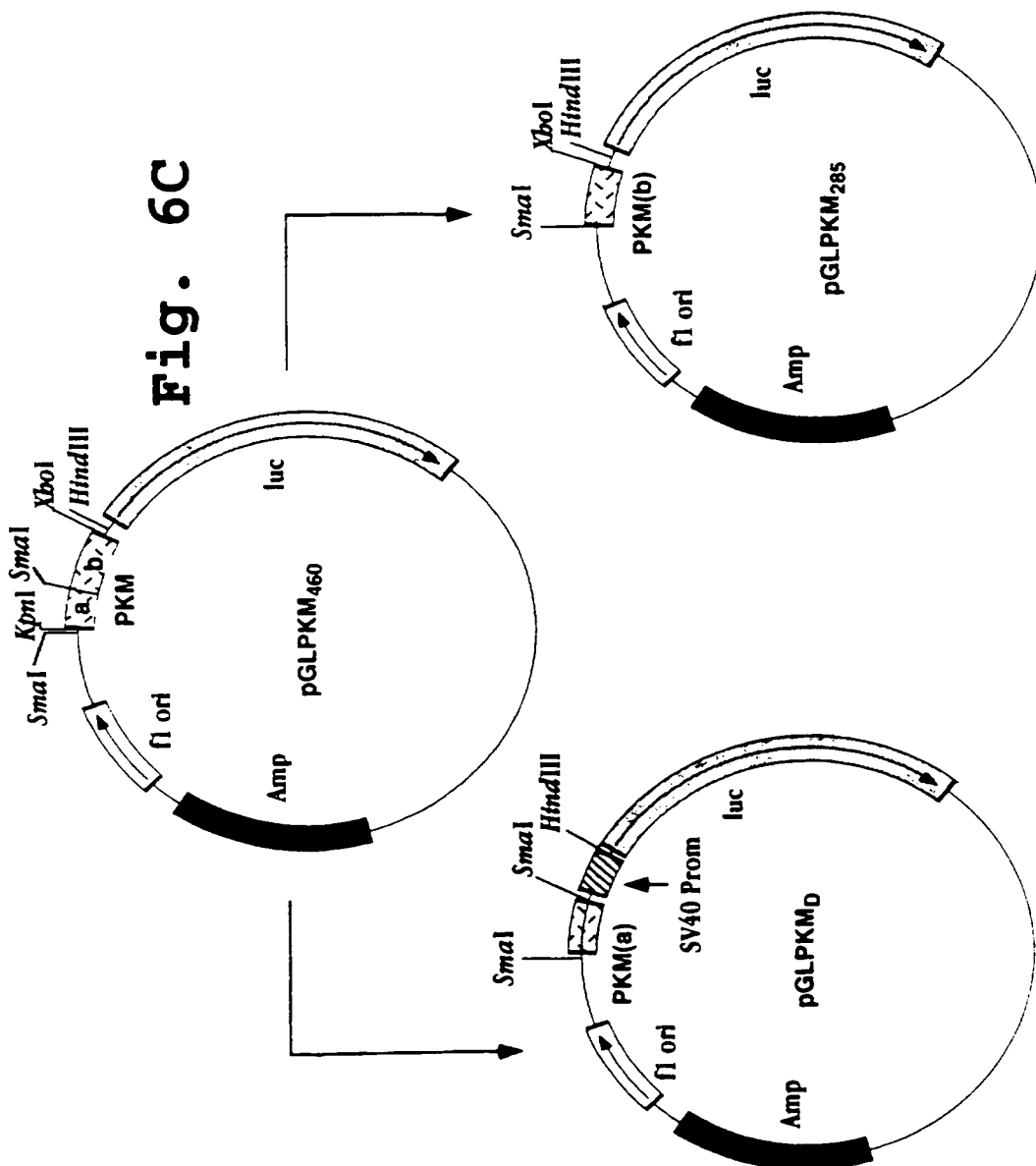
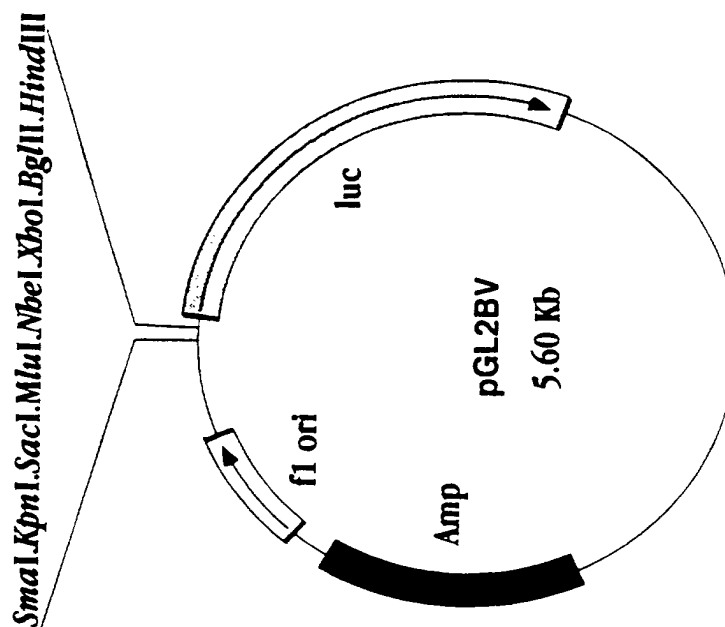
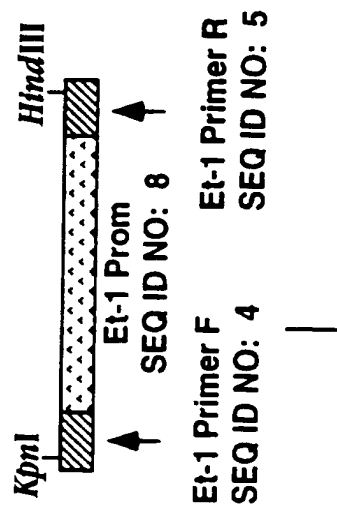


Fig. 6C

Fig. 6E

Fig. 6D

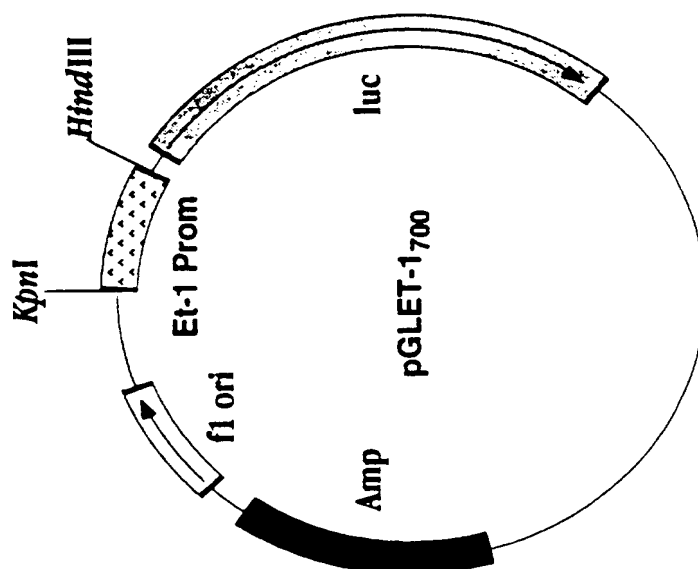
9/10

Fig. 7B**Fig. 7A**

To Fig. 7C

10/10

Fig. 7C



INTERNATIONAL SEARCH REPORT

International Application No.

PCT/IB 95/00996

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/11 C12N15/67

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROCEEDINGS OF THE ACADEMY OF SCIENCE, vol. 90, 1993 pages 3928-3932, A. MADAN ET AL. 'A 24-bp sequence 3' to the human EPO gene contains a hypoxia-responsive transcriptional enhancer' *see the whole article*	1-20
X	PROCEEDINGS OF THE ACADEMY OF SCIENCE, vol. 90, 1993 pages 4304-4308, G.L. WANG ET AL. 'General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia' *see the whole article*	1-20

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
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- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

1 April 1996

Date of mailing of the international search report

22.04.96

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Fax (+ 31-70) 340-3016

Authorized officer

Marie, A

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/IB 95/00996

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>BLOOD, vol. 82, no. 3, 1993 pages 704-711, I. BECK ET AL. 'Characterization of hypoxia responsive enhancer in the human EPO gene shows presence of hypoxia inducible 120 kd nuclear DNA-binding protein in EPO-producing and nonproducing cells' *see the whole article*</p> <p style="text-align: center;">---</p>	1-20
X	<p>MOLECULAR AND CELLULAR BIOLOGY, vol. 12, no. 12, 1994 pages 5447-5454, G.L. SEMENZA ET AL. 'A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human EPO gene enhancer at a site required for transcriptional activation' *see the whole article*</p> <p style="text-align: center;">---</p>	1-20
X	<p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE, vol. 91, 1994 pages 9496-9500, J.D. FIRTH ET AL. 'Oxygen regulated control elements in the PGK 1 and LDH-A genes' *see the whole article*</p> <p style="text-align: center;">---</p>	1-20
X	<p>CELLULAR AND MOLECULAR BIOLOGY RESEARCH, vol. 40, no. 1, 1994 pages 35-39, A. MINCHENKO ET AL. 'HYPOXIA REGULATORY ELEMENTS OF THE HUMAN vegf GENE' *see the whole article*</p> <p style="text-align: center;">---</p>	1-20
X	<p>FASEB JOURNAL, vol. 8, no. 4-5, 1994 page A128 B.J. MURPHY ET AL. 'Metallothionein IIa is upregulated by hypoxia in human squamous carcinoma cells' *see the whole abstract*</p> <p style="text-align: center;">---</p>	20
X	<p>CANCER RESEARCH, vol. 54, 1994 pages 5808-5810, B.J. MURPHY ET AL. 'Metallothionein IIa is up regulated by hypoxia in human A431 squamous carcinoma cells' *see the whole article*</p> <p style="text-align: center;">-----</p>	20

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